HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 9 JANUARY, 1935 No. 1

THE CALIFORNIA APPARATUS FOR RESPIRATION TRIALS WITH LARGE ANIMALS[1]

MAX KLEIBER[2]

INTRODUCTION

ENERGY METABOLISM is one of the fundamental vital functions. Its measurement is therefore the key to a great many physiological problems. In the field of animal nutrition, the determination of the energy exchange enables the investigator to express the effect of food in terms of gain or loss of chemical energy instead of using the less reliable criterion, body weight. The main objects of investigation in this field are determination of relative food values; measurement of the effect of environmental conditions, methods of feeding, and quality of food on food utilization; and determination of the relative values of animals as utilizers of food, with a view to securing data which may become important for genetic purposes.

This paper discusses the problems involved in the measurement of energy metabolism in large animals and describes the respiration apparatus in operation at the Branch of the College of Agriculture, University of California, Davis, California. The apparatus was constructed in 1929 and 1930, and more than three hundred measurements of the 24-hour metabolism of cows and heifers have been conducted up to this time. The apparatus may therefore be regarded as completed for its present purpose. It will, however, be further developed as experience with its operation grows and as new problems calling for its use may demand. In order to simplify future reports on results obtained with this apparatus, it seems desirable to describe it in its present state, together with the procedure followed in respiration trials with cattle at this experiment station.

[1] Received for publication March 9, 1934.

[2] Associate Animal Husbandman in the Experiment Station.

GENERAL PRINCIPLES IN MEASURING METABOLISM

Direct and Indirect Calorimetry

As any chemical process is related to a definite transformation of energy, one could calculate the energy metabolism if the complete chemical metabolism were known. Although the chemical processes in the living animal are not yet completely understood, one need not know every detail of them in order to calculate the energy metabolism; only the initial and final states need be known. If a given amount of material is transferred from a certain initial to a certain final state (for example, crystalline saccharose and O_2 to water, CO_2, O_2, and additional heat with initial and final state at given temperature and pressure), the sum of all transformations of chemical energy into heat is definite—that is, independent of any variations in the intermediary processes. This law of the initial and final states, also called the law of Hess, who demonstrated it in 1840 (Chwolson, 1922) has been applied to the energy transformations in the animal, especially by Rubner (1894), and Atwater and Benedict (1899). Only the two authors last named really carried out work trials and were thus enabled to establish as valid for living beings the law of conservation of energy, which includes the relation of mechanical work to heat, and of which the law of Hess, earlier discovered, is only a part.

From Hess's law, one may formulate for the resting animal:

$$U_f = U_e + U_b + H, \tag{1}$$

where U_f = chemical energy in food, determined as heat of combustion
U_e = chemical energy in excreta (feces, urine, methane, milk)
U_b = chemical energy in produced body substance
H = heat production of animal

The heat of combustion (U_f) is determined in the calorimetric bomb at constant volume and the animal heat (H) under practically constant pressure. Therefore it might be questioned whether the work of expansion or the negative work of contraction involved in this oxidation process in the animal should be considered. This work is small in comparison with the heat of combustion, as already mentioned by Borsook and Winegarden (1930, p. 561). It is zero for the oxidation of carbohydrates and amounts to 0.2 per cent of the heat of combustion when tripalmitin is oxidized.

In order to establish Hess's equation as correct for the living animal, one must measure the chemical energy of the food, excreta, and built-up or broken-down body substance, and the heat produced by the animal.

The chemical energy of the produced body substance can ordinarily not be measured directly, but is calculated from the determination of the carbon and nitrogen metabolism. Since, however, this calculation itself depends on the validity of Hess's law, this law can be proved strictly only if the change in body substance (U_b) is negligible.

Measurement of heat production (H) is called direct calorimetry. The heat production may be determined by *indirect* calorimetry, on the basis of the equation given above, provided the three other terms are known.

According to Hess's law, direct and indirect calorimetry should give equal results for the animal's heat production. The apparatus used to check the validity of this law in animal metabolism is called a respiration calorimeter. The ideal method would be to make all metabolism experiments in such a respiration calorimeter so that the check on the reliability of the calculation for each result would be at hand. The high cost of the apparatus, its complicated construction, and its difficult operation are probably the main reasons for there being, at present, only one respiration calorimeter in operation in the world for animals the size of cattle—namely, that built by Armsby at Pennsylvania State College in 1903 (Armsby, 1904). Such an apparatus is necessary for answering the fundamental question of whether or not methods of calculating the energy metabolism from the chemical metabolism are correct under different conditions—for example, for positive and negative changes in body substances, for animals producing CH_4, and for those secreting milk.

In cases, however, where the validity of Hess's law and the reliability of certain calculations are not themselves under investigation and are assumed to be established, the metabolism study may be simplified. One may measure the chemical metabolism, obtain U_f, U_e, and U_b, and calculate from these data the energy metabolism. This is done in an ordinary respiration experiment. Or one may omit the respiration trials (that is, the determination of the gaseous exchange), measure the heat production, and calculate the changes in chemical energy of the body substance, as with the large Cambridge calorimeter of Capstick (1926).

Theoretically it is simpler to determine the heat of combustion of the food (U_f) and of the excreta (U_e), and the heat production (H), and then calculate the change of chemical energy of the body (U_b) than to determine first the carbon in the food and excreta, including CO_2 and CH_4, then calculate the change in carbon stored in the body, and finally from this result derive U_b by indirect calorimetry. At present, however, determination of the carbon balance seems much easier and less expensive than direct measurement of the heat production (H) of the animal.

To construct a chamber sufficiently air-tight for a respiration trial seems easier than to construct a large calorimeter that is adiabatic or at least is heat-tight enough for a calorimetric measurement. As the Cambridge calorimeter shows, such an apparatus can be built for certain purposes within reasonable cost, though the difficulty may be increased if trials are to be carried out for several weeks with the necessary equipment for feeding, sampling excreta, and milking cows.

For obtaining results, indirect is as satisfactory as direct calorimetry if the heat production itself is not the main problem. In almost all meta-

TABLE 1

Heat Equivalent per Liter O_2 and CO_2*

Substance	O_2 consumption per gram	CO_2 production per gram	Respiratory quotient, $\frac{CO_2}{O_2}$	Heat production per gram	Heat production		Deviation from average of heat production per liter gas			
					Per liter O_2	Per liter CO_2	Calories		Per cent of average	
							O_2	CO_2	O_2	CO_2
	cc	*cc*		*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*per cent*	*per cent*
Protein	966.3	773.9	0.801	4.3160	4.485	5.579	−0.254	−0.173	−5.37	−3.01
Fat	2,019.3	1,427.3	0.707	9.4610	4.686	6.629	−0.053	+0.877	−1.14	+15.3
Starch	828.8	828.8	1.000	4.1825	5.047	5.047	+0.308	−0.705	+6.50	−12.3
Average	.....	.....	.....	.....	4.739	5.752	.....	.....	.....	.....

* In this publication heat is expressed in kilogram calories (abbreviated Cal.), one kilogram calorie being the amount of heat which raises the temperature of 1 kilogram of water from 14.5° C to 15.5° C. In recent work this amount of heat is defined as 4,184 joule. Some of the data used may be based on the older definition of the calorie—Regnault's calorie or the mean calorie; the resulting differences are, however, less than 1 per cent and do not affect the conclusions drawn from these data. Armsby (1922, p. 220) recommends the mean calorie but gives as the equivalent in his table on the next page 4.184×10^{10} ergs which corresponds to the definition given above. One therm = 1,000 kilogram calories.

bolic research connected with agriculture, not the heat production but the change in body substance is primarily interesting. For a study of these changes, the measurement of the chemical metabolism is as satisfactory as directly measuring the heat output of the animal, if not more so. In principle, the energy metabolism can be calculated from the chemical metabolism; but the reverse calculation is impossible. The determination of the chemical metabolism thus provides more information than does that of the energy metabolism alone. The respiration chamber would therefore remain an important piece of equipment for metabolism studies, even if the direct calorimetry could be carried out as easily and accurately as the determination of the carbon balance.

Relative Importance of CO_2 and O_2 Determination

The indirect calorimetry may be based upon the CO_2 production or the O_2 consumption or both. Either CO_2 or O_2 may be the more important under certain circumstances. If the respiration experiment is principally intended to determine indirectly the heat production of the ani-

mal, measuring the O_2 consumption is preferable to measuring the CO_2 production because the heat production per liter$_s$[3] of O_2 consumed varies less than that per liter$_s$ of CO_2 produced, as shown by table 1, the first seven columns of which are given by Loewy (1926, p. 273).

As mentioned above, in most agricultural experiments the heat production of the animal has but a secondary importance. The main question is the change in body substance. This change may, according to Henneberg's conception of the schematic body of an animal (Armsby, 1908), be taken as occurring in fat and protein if the water and mineral substances are not considered. A possible change in the glycogen content of the body is neglected in this simplification by calculating this change as fat. The error that occurs by calculating 1 gram of carbon in glycogen as 1 gram of carbon in fat, when estimating heat production, appears in table 2.

TABLE 2

KILOGRAM CALORIES PER GRAM CARBON IN GLYCOGEN AND FAT

From	Carbon per gram	Heat per gram	Heat per gram carbon
	grams	*Cals.*	*Cals.*
Glycogen	0.444	4.19	9.4
Fat	0.765	9.46	12.4
Difference	.....	.....	3.0

The amount of glycogen in the body is only a few grams per kilogram of total weight. For experiments of several weeks' duration, the error introduced by disregarding the changed glycogen content of the body is therefore not considerable. In a fattening trial of four weeks' duration, if an ox gains daily 800 grams of body fat, as did Kellner's steers (Kellner and Köhler, 1900, p. 52) it will gain in 28 days about 22 kg of fat, equal to 210 therms. The steer may be assumed to gain or lose during that time 1 kg of glycogen—a maximal estimate, being about 50 per cent of its total glycogen.[4]

One kg of glycogen has a heat of combustion of 4.19 therms. It contains 444 grams of carbon, which would represent 5.51 therms if calculated according to the simplification with Henneberg's schematic body, as fat with 12.4 Cals. per gram carbon. The error of calculation that

[3] The subscript $_s$ is used in this paper to indicate the reduction of a gas volume to standard conditions, that is, volume of dry gas at a pressure of 760 mm Hg and at 0° C.

[4] Armsby (1922, p. 61) estimates the glycogen content of a steer of 1,200 pounds as 2.2 kg, assuming for the liver a glycogen content of 10 per cent; for the muscles, 4 per cent.

arises from neglecting this maximum change in glycogen and from calculating the total gain of carbon besides protein as fat, amounts thus to 5.51 — 4.19 = 1.32 therms, or 0.6 per cent of the result of a four weeks' trial.

The same calculation would apply to a dairy cow yielding 210 therms of milk in 28 days, which, with 0.7 therm per kg of milk, is $\frac{210}{0.7} = 300$ kg total or 11 kg (24 pounds) per day. As the coefficient of variation of Kellner and Köhler's eleven results (1900, p. 450 and 451) for determining the net energy of starch is ± 11 per cent, a possible maximum error of 0.6 per cent is negligible; and consequently a constant glycogen content of the body may, according to Henneberg's scheme, be assumed for experiments of long duration. In such experiments, the findings of Benedict and Ritzman (1923, p. 48) are still valid. These authors state:

> While, therefore, we are in no sense disposed to minimize the advisability and great desirability of simultaneous measurements of carbon dioxide production, oxygen consumption, methane elimination, and particularly direct heat measurements, we have come to the conclusion, based upon Professor Armsby's remarkable contributions to the gaseous metabolism of animals, that an apparatus which measures accurately, rapidly, and inexpensively carbon dioxide production, has a proper field in experiment station research.

This statement suggests that today the determination of CO_2 alone is even better justified than in 1923, for since then Armsby's work has been carried on by Forbes and collaborators; Benedict and Ritzman have added to their determination of the CO_2 production of steers that of O_2 consumption; and Möllgard has obtained valuable data on dairy cows for both CO_2 production and O_2 consumption. Thus the basis for calculating the metabolism on CO_2 production alone is wider than eleven years ago.

In prolonged experiments for determining the nutritive value of food or the animal's efficiency, and in fact in the solution of most feeding problems, the measurement of the CO_2 production alone as the basis for the carbon balance in addition to the nitrogen balance is satisfactory. It is even preferable to the determination of the O_2 consumption alone, because the CO_2 production allows one to determine directly the amount of carbon stored or lost by the animal. This result will be independent of any changes in the factors for the energy equivalent of $CO_2$and O_2 that may be indicated by future research in the field of indirect calorimetry.

In brief experiments, however, the simplification with the schematic body is not applicable, because a change in the glycogen deposit may play an essential part in the metabolism. Zuntz (1926, p. 435) calculates,

for example, that one of his horses used up during 95 minutes of work 120 grams of fat and 525 grams of glycogen, the latter being one-fourth of the total glycogen deposit.

The heat of combustion of the substance actually used may be calculated as in table 3.

If the glycogen had been neglected and the total carbon used had been calculated as carbon from fat with a heat of combustion of 12.4 Cals. per gram of carbon, the heat of combustion of the material used would have been calculated as $12.4 \times 325 = 4{,}030$ Cals. instead of 3,340 Cals., which would thus have been an error of + 20 per cent of the result.

TABLE 3

HEAT FROM GLYCOGEN AND FAT IN SHORT EXPERIMENT

Substance used	Carbon per gram	Carbon in substance used	Heat per gram substance	Heat of substance used
	grams	*grams*	*Cals.*	*Cals.*
120 grams fat	0.765	92	9.5	1,140
525 grams glycogen	0.444	233	4.2	2,200
Total	.....	325	.....	3,340

In short experiments (those of only a few hours' duration) neglect of a change in the glycogen content of the body may thus introduce considerable error. In these cases, determining the O_2 consumption alone is preferable to determining the CO_2 production alone.

THE RESPIRATORY QUOTIENT (R.Q.)

The safest procedure is to determine both CO_2 production and O_2 consumption, because the ratio $\frac{CO_2 \text{ produced}}{O_2 \text{ consumed}}$, the R.Q., may be used to calculate the amounts of fat and carbohydrate burned. The calculation may be carried out as follows:

The number of mols of CO_2 produced from the combustion of a mixture of fat and carbohydrate containing C_F grams of carbon in fat and C_Z grams carbon in carbohydrate would be $\frac{1}{12} C_F + \frac{1}{12} C_Z$ because 1 gram of carbon is $\frac{1}{12}$ mol, and one mol of carbon corresponds to one mol of CO_2.

According to the equation for the respiratory quotient of fat,[5]

$$(\text{R.Q.})_F = \frac{(CO_2)_F}{(O_2)_F} = 0.707, \qquad (2)$$

[5] See Krogh, 1916, p. 6.

the amount of O_2 used up in terms of mols for the combustion of fat, $(O_2)_F$, is

$$(O_2)_F = \frac{(CO_2)_F}{0.707}.$$

As

$$(CO_2)_F = \frac{C_F}{12},$$

then

$$(O_2)_F = C_F \frac{1}{12 \times 0.707}. \tag{3}$$

For the combustion of carbohydrate, the number of mols of O_2 used equals the number of mols of CO_2 produced, and hence the respiratory quotient for a carbohydrate, $(R.Q.)_Z = \frac{(CO_2)_Z}{(O_2)_Z} = 1$. The amount in mols of O_2 used for the combustion of a mixture with C_F grams of carbon in fat and C_Z grams of carbon in carbohydrate is thus

$$O_2 = C_F \times \frac{1}{12 \times 0.707} + C_Z \times \frac{1}{12}. \tag{4}$$

The following calculation may therefore be carried out for the R.Q. of a mixture of carbohydrates and fats:

$$R.Q. = \frac{CO_2}{O_2} = \frac{\left(C_F \times \frac{1}{12}\right) + \left(C_Z \times \frac{1}{12}\right)}{\left(C_F \times \frac{1}{12 \times 0.707}\right) + \left(C_Z \times \frac{1}{12}\right)}$$

or

$$R.Q. = \frac{C_F + C_Z}{\left(C_F \times \frac{1}{0.707}\right) + C_Z} \tag{5}$$

If C'_Z represents the percentage of carbon as carbohydrate carbon, and C'_F the percentage of carbon as fat carbon,

then $$C'_Z = 100 - C'_F \tag{6}$$

By substitution of this value in the equation 5, one obtains

$$R.Q. = \frac{C'_F + 100 - C'_F}{\left(C'_F \times \frac{1}{0.707}\right) + 100 - C'_F} \tag{7}$$

and finally

$$C'_F = 241\left(\frac{1}{\text{R.Q.}} - 1\right) \tag{8}$$

For the partition of energy from metabolized fat and carbohydrates one may write:

$$U_F = C_F \times f \tag{9}$$

which means that the heat derived from fat, U_F, is the heat per gram of carbon in fat, f, times the number of grams of carbon in the fat, C_F, because a given amount of fat contains a definite amount of carbon and has a definite heat of combustion. In metabolism, to be sure, many different fats must be considered; if, however, a general calculation is to be possible, one must abstract a typical representative with a well-defined relation of chemical composition and heat of combustion.

Similarly, one may formulate:

$$U_Z = C_Z \times z \tag{10}$$

where U_Z = heat of combustion of a certain amount of carbohydrate
C_Z = grams of carbon in that amount of carbohydrate
z = heat of combustion of the carbohydrate per gram of carbon.

Substituting the values

$$C_F = \frac{1}{f} \times U_F$$

and

$$C_Z = \frac{1}{z} \times U_Z$$

in the equation 5 for R.Q. above, one obtains:

$$\text{R.Q.} = \frac{\left(U_F \times \frac{1}{f}\right) + \left(U_Z \times \frac{1}{z}\right)}{\left(U_F \times \frac{1}{f} \times \frac{1}{0.707}\right) + \left(U_Z \times \frac{1}{z}\right)} \tag{11}$$

If in the combustion of a mixture of fat and carbohydrate, U'_F stands for the percentage of heat obtained from fat and U'_Z for the percentage of heat obtained from carbohydrate, then one may write:

$$U'_Z = 100 - U'_F \tag{12}$$

Thus

$$\text{R.Q.} = \frac{\left(U'_F \times \frac{1}{f}\right) + \left[(100 - U'_F) \times \frac{1}{z}\right]}{\left(U'_F \times \frac{1}{f} \times \frac{1}{0.707}\right) + \left[(100 - U'_F) \times \frac{1}{z}\right]} \tag{13}$$

and further

$$U'_F = \frac{100(1-\text{R.Q.})}{\left[\text{R.Q.}\left(\frac{z}{0.707f}-1\right)\right]+1-\frac{z}{f}} \tag{14}$$

If starch, a gram of which contains 0.444 gram of carbon and has a heat of combustion of 4.1825 Cals. (Loewy, 1926, p. 272), is taken as a representative of carbohydrate, the heat production per gram of carbon, z, is 9.411 Cals.

For fat, with 0.7653 gram of carbon and heat of combustion of 9.461 Cals. per gram, each gram of carbon corresponds to a heat production, f, of 12.36 Cals.

If these figures are substituted for the percentage energy from fat, the equation becomes:

$$U'_F = \frac{100(1-\text{R.Q.})}{(0.077\times\text{R.Q.})+0.238} \tag{15}$$

The result of this calculation appears in table 4.

For an approximation, the denominator of the formula given above, (0.077 R.Q.) + 0.238 may be taken as a constant; consequently, the proportion of calories from fat to the total calories from fat and carbohydrate might be regarded as a linear function of the respiratory quotient. This seems to have been the procedure of Williams, Riche, and Lusk (1912) for obtaining their data, which have been copied by Carpenter (1921, p. 104) for his biochemical tables.

The comparison between the results of the calculation, as developed above, and the linear interpolation appears in table 4.

TABLE 4

Proportion of Calories from Fat

Respiratory quotient	Calculated from formula	Calculated as linear function* of R.Q.	Difference
	per cent	*per cent*	*per cent*
0.750	84.4	85.0	0.6
0.800	66.7	68.0	1.3
0.850	49.4	51.0	1.6
0.900	32.5	34.0	1.5
0.950	16.0	17.0	1.0
1.000	0.0	0.0	0.0

* From Carpenter, 1921.

The difference is not serious. When, however, the percentages are given with a decimal place, the deviation from the linear function should not be neglected.

The heat of combustion per liter O_2 at standard conditions must, according to Zuntz and Schumburg (1901, p. 361) be a linear function of the respiratory quotient. Although it is not quite clear, *a priori,* why this should be so, it is worth while to know that by calculation this linear relation can be proved to exist. The result of this calculation is

$$U_o = 85.43 + 27.51 \times \text{R.Q.} \qquad (16)$$

where U_o stands for the heat of combustion in Cals. per mol of O_2.

For a respiratory quotient of 1 (carbohydrate alone), the heat per mol of oxygen is 112.94 Cals.; for one of 0.707 (fat alone), 104.88 Cals. Per gram of O_2, the heat of combustion is, for carbohydrate and fat, respectively, 3.529 Cals. and 3.277 Cals.; and per liter$_s$ of O_2, using a density of O_2 as 1.4290 grams per liter$_s$ (Pickering, 1928, p. 3), the heat production is 5.044 Cals. if R.Q. = 1, and 4.684 Cals. if R.Q. = 0.707. These are the values given by Zuntz and Schumburg and used by Lusk and Carpenter.

The values per mol of CO_2 may be obtained by dividing the figures per mol of O_2 by R.Q. For a respiratory quotient of 1, the heat production per mol of CO_2 is thus 112.94 Cals., and for one of 0.707 it is 148.35 Cals.

The density of CO_2 is 1.9769 grams per liter (Pickering, 1928, p. 3). We may, however, reasonably assume that CO_2 in the small concentrations in which it is present in air from respiration chambers (partial pressure only 1/100 atmosphere) acts as an ideal gas, as does oxygen, containing 0.04466 mol per liter$_s$ and consequently having a density of 1.9652 grams per liter$_s$.

This value, given in Carpenter's tables, should be distinguished from the correct density of CO_2 at normal conditions. It should be designated as the density of diluted CO_2.

Using a density of 1.9652 grams per liter$_s$, the heat equivalent of CO_2 is 5.044 Cals. per liter$_s$ for a respiratory quotient of 1.0, and 6.625 Cals. for a respiratory quotient of 0.707.

Check of Results

The heat production of the animal may be calculated:

1. On the basis of the CO_2 production alone, by determining the carbon balance and the nitrogen balance, thus calculating how much fat and protein the animal has lost or gained, neglecting a possible change of glycogen in the animal's body.

a) The energy content of food and excreta is calculated from the chemical analysis; or

b) The energy content of food and excreta is measured directly in a bomb.

2. On the basis of O_2 consumption alone, by an approximation, using an average heat equivalent for one liter of O_2.

3. On the basis of the CO_2 production and the O_2 consumption, by using a better-defined heat equivalent for one liter of either gas—on the basis of the nitrogen-free R.Q.

If *a* and *b* of calculation 1 give the same result, this is a check for the standard figures used for calculating the heat of combustion of food and excreta from the chemical analysis. This check is very desirable. The determination of the carbon content and the measurement of the heat of combustion in food and excreta may be combined by using for the calorimetry a bomb with a double valve that allows one to drive the air remaining from the combustion over an absorber for CO_2.

If the same result is obtained by calculation 1, neglecting glycogen, and by calculation 3, using the nitrogen-free R.Q., then the conception of the schematic body can be applied to this case; or the standard figures for the heat equivalent per liter of O_2 or CO_2 at a certain R.Q. are confirmed.

The agreement between two results of indirect calorimetry cannot, however, be taken as proof that the metabolism of an animal is in agreement with the law of conservation of energy, as might be concluded from Möllgaard's statements (1929, p. 44). An experiment in which the two kinds of calculation of the heat production strictly agree might still not conform with the law of conservation of energy, since it might be argued that the animal could produce outside work without using up energy, and that one would be unable to notice this fact in a respiration trial. Or, on the other hand, the two results of the indirect calorimetry may not check; one would conclude from this result, not that the metabolism of the animal contradicted the law of conservation of energy, but rather that the factors used for calculating the heat indirectly were incorrect in this particular case.

Not even Hess's law, the thermochemical part of the law of conservation of energy, can be proved by indirect calorimetry alone, which, on the contrary, is based on the assumption that Hess's law is valid. In order to test whether or not the metabolism of an animal agrees with Hess's law, a trial in a respiration calorimeter is necessary; that is, measurement of the chemical metabolism must be combined with direct measurement of the heat production. In order to study the validity of the law of conservation of energy for animal life, one should determine the external work done by the animal, in addition to calculating the chemical metabolism and the heat production, as has been done by Atwater and Benedict (1899, 1900–1902, 1903).

Although the validity of the fundamental law of thermodynamics and

thermochemistry cannot be tested by means of indirect calorimetry, it is very valuable to carry out the necessary determinations for calculating the heat production of the animal on the basis of the carbon balance, besides using the R.Q. (calculations 1 and 3) as a check on the data in the calculation.

Choice of the System

Krogh (1916, p. 20) classifies the methods for studying the respiratory exchange in two main groups: the methods in which the animals are enclosed in a respiration apparatus, and those in which only the respiratory organs are connected with the apparatus. The latter methods are suitable for brief experiments and require, if applied to animals, tracheotomy (Möllgaard and Anderson, 1917, p. 44), or an air-tight muzzle (Brodie, 1928). One of the first-mentioned methods has therefore been chosen.

Within this enclosure method, two different types of apparatus are in use: the closed-space type and the open-air-current system. The closed-space type, of which Regnault and Reiset's apparatus is the classical representative, must be absolutely air-tight, the temperature and humidity within it well defined. These requirements can easily be met for small apparatuses suitable for determining the respiration of small animals or of tissues (Krogh, 1916, p. 23). An apparatus for large animals designed on this principle is, however, very difficult and expensive to operate. The 80 cu. m chamber of Zuntz is said to be extremely hard to keep air-tight and to maintain at a definite volume, since large forces act on the walls if the air pressure changes but slightly. Also, an error of 1° C in temperature would cause an error of 240 liters O_2 consumption, or 16 per cent of a cow's daily metabolism. The apparatus is very expensive to operate because one day's trial with cattle requires approximately 50 to 100 kg KOH.

For these reasons, the open-air-current system, known as the Pettenkofer type, has been found more suitable for the California Experiment Station. The original disadvantage of this apparatus—that one could determine only the CO_2 production but not the O_2 consumption of the animal—has been overcome by Sondén and Tigerstedt (1895), who substituted for the steady absorption of the CO_2 of an aliquot air current, the collection of a composite sample that could later be analyzed for both CO_2 and O_2 in the Petterson-Sondén gas analysis apparatus (Petterson, 1886). The old Pettenkofer method was therefore combined with that of Tigerstedt for obtaining checks on the results; and this respiration apparatus would be classified as a combined Tigerstedt and Pettenkofer open-air-current enclosure apparatus.

THE RESPIRATION APPARATUS AT DAVIS

General Scheme of the Apparatus at the University of California

A schematic sketch of the California apparatus appears in figure 1. For the sake of simplicity, only one of the two chambers is drawn.

During the upward movement of one of the large aspirator pipettes (there are actually four such pipettes in the same basin) out of the

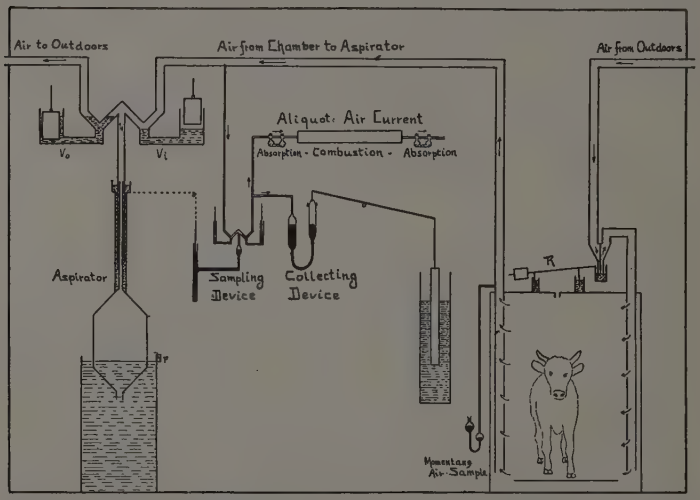


Fig. 1.—General scheme of the Davis apparatus: *P*, productimeter; *R*, regulator for air pressure; *Vi*, inlet valve of aspirator; *Vo*, outlet valve of aspirator; *W*, water basin.

waterbasin, *W*, the valve *Vi* is open (as shown in the sketch), and air is thus sucked from the chamber into the pipette. Before the pipette moves downward the valve *Vi* is closed and the valve *Vo* opened so that, as the pipette moves downward, the amount of air that it contains is pushed outdoors. A productimeter records the number of movements of the pipettes. This number and the volume of water flowing out of the pipette during the upward movement are used for calculating the amount of air sucked out of the chamber in a given time. From the air leaving the chamber a momentary sample is taken (fig. 1) and analyzed in order to determine the amount of CO_2 and O_2 contained in the chamber at a definite moment. Such a momentary sample is always taken at the beginning and at the end of an experimental period, which generally lasts 12 to 24 hours.

In the central part of the sketch is shown the sampling device built according to the same principle as the aspirator. During each upward movement of one aspirator pipette (two of these pipettes are working alternately for each chamber), 1 cc of the air flowing from the chamber to the aspirator pipette is drawn over mercury into a glass pipette. During the downward movement of the aspirator pipette, this sample is pushed to a bulb in which the small samples are collected over mercury, forming the composite sample for a whole period. This sample is later analyzed for CO_2 and O_2. Every time that the 1-cc sample is taken and stored in the collecting bulb, a 20-cc sample is also drawn by the same device and pushed over an absorbing system for CO_2, whence it passes through a combustion tube and again over an absorber for CO_2.

The amount of CO_2 leaving the chamber is determined in two ways:

1. By multiplying the amount of air sucked out, as indicated by the productimeter and the volume of the aspirator pipette, by the concentration of CO_2 in the composite sample, as determined by gas analysis.

2. By multiplying the amount of CO_2 determined by titration in the first absorber of the aliquot current, by the ratio of the volume of the two aspirator pipettes to the 20-cc sampling pipette.

The O_2 leaving the chamber is determined on the basis of the analysis of the composite sample and the amount of air sucked out.

The amount of CH_4 leaving the chamber is calculated from the amount of CO_2 found by titration in the second absorber and from the ratio of the volume of the sampling pipette to the volume of two aspirator pipettes.

How the respiratory exchange is calculated on the basis of these data will be discussed in a separate section (p. 51).

The Chamber

General Principles.—The comfort of the cow, necessary for reliable results, has been an important guide in constructing the respiration chamber. The cow, as a typical herd animal, tends to be nervous if placed alone in unaccustomed surroundings. This excitement may so greatly influence the metabolism that all precautions for an accurate chemical determination will be almost useless with regard to the biological result. This consideration was one reason for building a double chamber in which two cows may be experimented upon at the same time, separated by an airtight partition but able to see each other through a window.

The double chamber has the further advantage of allowing pair trials—that is, the testing of two animals closely similar in their internal as well as the external conditions, except for the one variable which

is the object of study. There may be influences on the metabolism that are not yet known. Perhaps, for example, atmospheric conditions besides temperature and humidity affect the rate of animal heat production. There may be periodic fluctuations of metabolic rate connected with unknown factors. Such influences on the metabolism, though unknown, may to a considerable degree be compensated by a control test carried out simultaneously with the main experiment, assuming that the unknown or unwanted variation of the conditions influences the experimental animal and the control in the same way.

Material.—The respiration chamber is built of tongue-and-groove "Oregon pine" (Douglas fir, *Pseudotsuga saxifolia*) fixed to a wooden frame of 2×4 inch studs. Three layers of building paper are applied, and outside this a waterproof mixture of a tar product and cement (Mortex) is fixed to the wooden wall by chicken wire. The outside is finished with cement plaster, and the surface painted. The roof is built of two layers of tongue-and-groove "Oregon pine" boards with three layers of building paper between them. The doors are also made of wood, sealed with rubber weather strips. The inside of the chamber is covered with aluminum paint, in order to decrease the porosity of the walls; and the floor is treated with a heavy coat of black asphalt, extending up the side walls for a few inches in order to secure an air-tight connection between wall and floor. On this asphalt, galvanized sheet iron is laid. The wooden walls of the chamber provide a good thermal insulation, which is an important factor in controlling temperature and humidity inside, particularly at extreme experimental temperatures.

Dimensions.—In a metabolism test, the smaller the chamber, the smaller the error caused by the limited accuracy of the gas analysis. This consideration led Jaquet to build the chamber of his apparatus for the investigation of human metabolism as "a cross between a brougham and a coffin" (Grafe, 1926, p. 335).

For measurements of at least 12 hours' duration, which are necessary in most agricultural problems, the volume of the chamber is less important. In an agricultural experiment station, however, problems may also arise requiring the measurement of metabolism in short periods—1 hour, for instance. This is the case if one is interested in the daily variations of metabolism, such as the duration of the specific dynamic action of food intake, or any other influence on metabolism caused by events of short duration that may affect the endocrine or the nervous system, such as mating or injection of substances.

The influence of the chamber volume on the concentration of CO_2 and O_2 in the outflowing air has been studied theoretically by the author (Kleiber, 1928, 1933*b*).

The following equations have been developed for concentration of the CO_2 in a momentary sample taken from the chamber at a certain time, disregarding the initial concentration and the concentration in the inflowing air.

$$C_e = \frac{\gamma}{L}\left(1 - e^{-\frac{L}{V}t}\right) \tag{17}$$

where

C_e = concentration of CO_2 in the end sample, caused by the CO_2 production in the chamber
γ = rate of CO_2 production in the chamber (liters per hour)
L = rate of ventilation (liters per hour)
V = volume of the chamber (liters)
t = time in hours
e = 2.71828 (basis of natural logarithms)

The equation for the concentration of CO_2 in the composite sample reads:

$$C_m = \frac{\gamma}{L}\left[1 - \frac{V}{Lt}\left(1 - e^{-\frac{L}{V}t}\right)\right] \tag{18}$$

where

C_m = concentration of CO_2 in the composite sample, caused by the CO_2 production inside the chamber.

The other symbols have the same significance as above.

The error in the result due to an error of the gas analysis of 0.003 per cent of the air volume may be calculated according to the following equation:

$$e_g^2 = \pm(0.00003 \times L \times t)^2 + (0.00004 \times V)^2 \tag{19}$$

in which e_g = error of the result (due to error in gas analysis) in liters CO_2 and the other symbols have the same meaning as in equation 17. According to this equation, for a cow producing 100 liters of CO_2 an hour and a ventilation rate of 10 cu. m of air an hour, the volume of the chamber should not exceed 10 cu. m if an error of 0.003 per cent in the gas analysis is to cause no larger error than 0.5 per cent of the result of a one-hour period.

Each chamber of the California apparatus has a volume of 11.4 cu. m. In this volume are included 0.9 cu. m for the feces and urine separating device and 0.46 cu. m for the feeding device. The room for the animal is 283 cm long, 168 cm wide, and 220 cm high. The dimensions in feet and inches are given on plan and section in figures 2 and 3.

Device for Feeding.—The feeding device is so constructed that the food may be supplied without opening the chamber. The feedbox is made of galvanized iron in the form shown in figure 3. It hangs on two hook-straps that are fastened to a heavy door constructed of wood and sheet

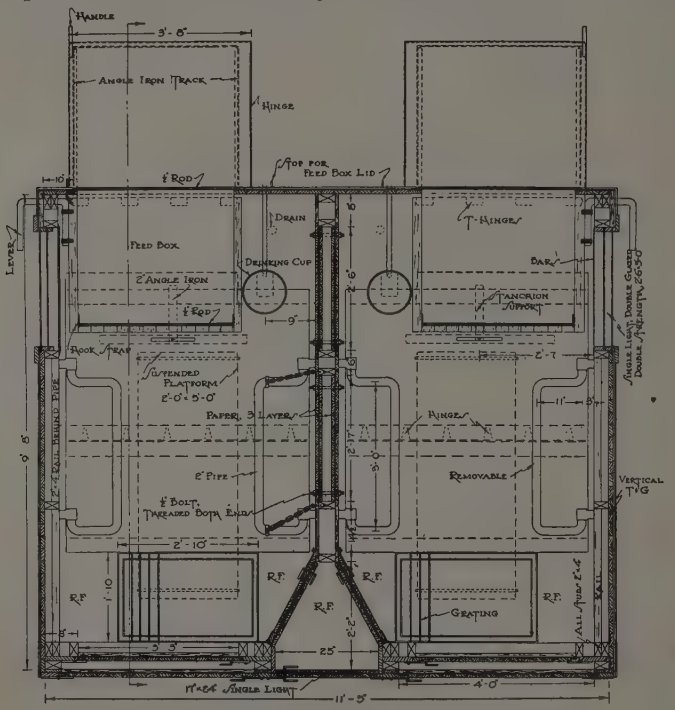


Fig. 2.—Plan of respiration chamber.

metal. This door is hinged to the floor of the chamber below an opening that allows the feedbox to pass through the front wall. The door is connected to a shaft that has one bearing in the side wall of the chamber and extends through it to the outside. The protruding end is square; and a long wrench attached to it acts as a lever to turn the shaft, and consequently the feedbox door, from outside. Fastened to the feedbox is an iron rod that guides it out along two tracks into a sheet-metal box built on to the front wall of the chamber. This sheet-metal box is provided with a cover in a water seal to make it air-tight and is covered with Celotex for insulation. (This insulation is not shown on the figures.)

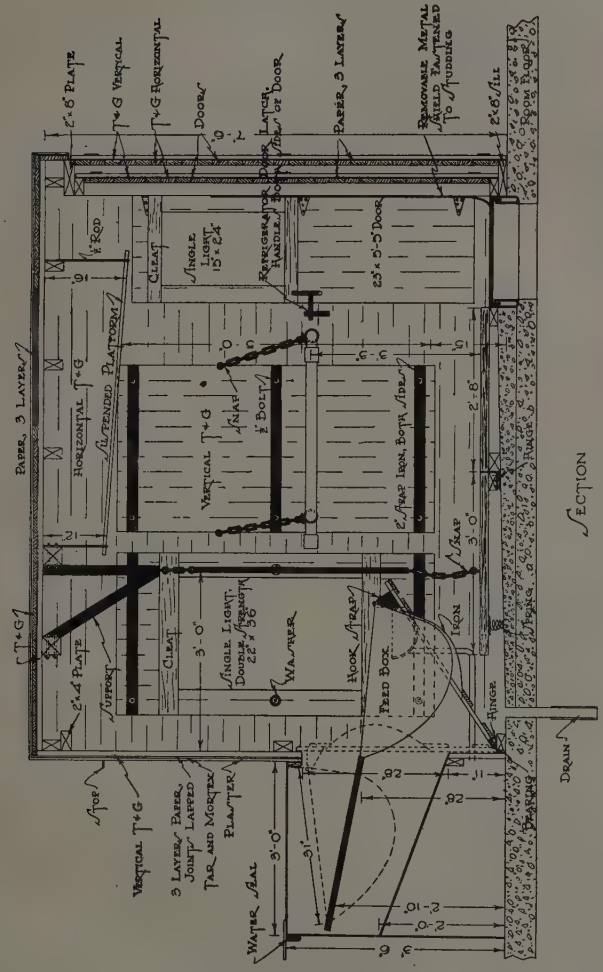


Fig. 3.—Section of respiration chamber. The spring platform shown here has been replaced by a platform mounted on inner tubes, as described on page 46.

To feed the cow, the lever is turned from outside, causing the feedbox to glide out through the opening in the front wall into the metal box, and at the same time moving the feedbox door so that it covers the opening completely. The cover of the outside metal box may then be opened to put the food into the feedbox. The outside box may then be closed again (the cover being put into the water seal) and the feedbox door turned back so that the feedbox glides into the chamber.

Drinking Water.—An ordinary automatic drinking cup is fastened in each chamber beside the manger. Before entering the drinking cup, the water passes a meter fixed outside in front of the chamber. A 15-gallon water tank is hung to the ceiling of the room in order to check the meter and to be used if distilled water is being given.

A three-way stopcock at the side makes it possible to connect the drinking cup with the 15-gallon tank or with the tapwater line, or to connect the tank with the tapwater line; but it is so arranged that all three connections can never be made simultaneously.

Feces-and-Urine-Separating Device.—For the separate collection of feces and urine, Ritzman's device (Ritzman and Benedict, 1929, p. 24) has been installed and enclosed air-tight with galvanized iron. Numerous water seals have been used so that the device may be opened and the feces container easily exchanged. A damper in the duct above the feces box may be closed for the time required to unhook the box with the feces and replace it with a clean one. This device allows the feces to be collected without interrupting the experiment.

The excrement-separating device is insulated against heat transfer toward the outside and is cooled inside by a pipe through which brine is circulated.

The Air-Conditioning System.—With regard to the accuracy of the result of the respiration trial as a function of the error of the gas analysis, one should not ventilate the chamber more than is necessary to keep the CO_2 concentration of the outflowing air at about 1 per cent, a level which has no influence on the behavior of the animal.

If the rate of ventilation is so low as to obtain a CO_2 concentration of 1 per cent, however, the cow would suffer from an excess of water vapor unless there were a means for dehumidifying the air inside the chamber. A fan (*F*) drives the air inside the chamber through an air channel above the cow (fig. 4). A radiator coil through which brine is circulated is installed in this channel. It is made of 15 meters of ¾-inch pipe with 700 fins which are 2 mm thick and 7.7 cm in diameter. The condensed water is collected in a container behind the cow. After leaving the condenser channel, the air passes an absorber for NH_3 (*N* in fig. 4) and electric strip heaters (*H*). The brine is cooled by an ice machine and is cir-

culated by a centrifugal pump *P*, driven by a ¼-hp. electric motor at a speed of 3,450 revolutions per minute. The brine flows first through the feces-and-urine-separating device, then through the condenser coil. In front of the chamber it passes a valve, *V*, which regulates the intensity of brine flow.

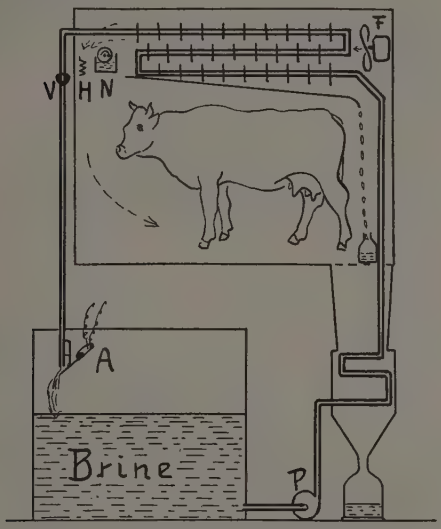


Fig. 4

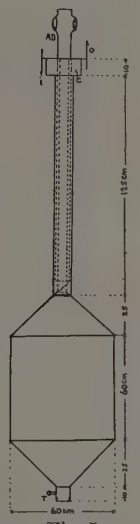


Fig. 5

Fig. 4.—Air-conditioning system: *A*, alarm device, makes alarm when brine flow is stopped; *F*, fan for circulating air in chamber; *H*, electrical strip heaters; *N*, absorber for NH_3; *P*, brine pump; *V*, valve for controling intensity of brine flow.

Fig. 5.—Aspirator pipette: *AD*, air duct; *C*, collar of pipette head; *i*, spring hook and pushing rod for mechanism of inlet valve; *o*, spring hook and pushing rod for mechanism of outlet valve; *T*, side outlet for calibration.

The electric current for the strip heaters is controlled by a mercury switch mounted on a balance on which are hung two 100-cc bulbs, containing mercury and connected at the bottom by a rubber hose. One bulb is sealed at the top and contains ether on top of the mercury; the other is open. By this device the air temperature in the chamber may be controlled within ± 2° C.

Device for Admitting an Attendant.—The double chamber is provided with a device that permits a man to enter without interrupting the experiment. The device is seen in the plan (fig. 2) as the triangular space at the rear end between the two chambers. The man first enters this narrow place, closing the outside door behind himself, and thence passes into either chamber, again closing the door.

The possibility of admitting a man is an advantage over the earlier chambers. It increases the comfort of the cow if the man to whom she is accustomed can enter and milk her and clean her in the ordinary way instead of milking her from outside by means of rubber gloves. It is often very helpful, during the experiment, if a man can enter the chamber to make small adjustments or repairs that otherwise might cause an interruption. The only limit to the duration of an experiment is thus the lack of exercise of the cows.

The device for admitting a man also has disadvantages.

1. The man's own respiration may affect the result.

2. The air of the triangular space may be mixed with the chamber; and when the man goes out, some of the CO_2 of the chamber escapes.

3. The doors are the weakest spot of the chamber as far as air-tightness is concerned.

The disadvantages are, however, not serious and are far outweighed by the advantages. As the respiratory exchange of the man is approximately one-fourth that of the cow, he could remain inside the chamber for an hour each day, and still the error introduced by neglecting his respiration would be only 1 per cent of the result. If one estimates the respiratory exchange of the man with an error of 10 per cent, the error of the experiment caused by his stay (one hour in twenty-four) in the chamber is only $\frac{1}{10}$ per cent. This calculation shows that in long experiments no special device is needed to take care of the man's respiratory exchange inside the chamber.

The second disadvantage is more serious because less easily controlled. It is hard to estimate the amount of CO_2 escaping in this way, but the maximal loss may be calculated. The volume of the triangular space is about 0.5 cu. m, or 5 per cent of the volume of the chamber. If, by opening of the inner door, the chamber air (supposed to contain 1 per cent CO_2) and the air in the anteroom were completely mixed, 5 liters of CO_2 would escape from the chamber to the triangular space. If, then, by opening of the outer door, this air were, in turn, again completely mixed with outside air, the total loss for one entrance of the man would be 5 liters of CO_2 or 0.3 per cent of the CO_2 production of a cow in 24 hours at maintenance. A stay of the man in the chamber for 20 minutes would about compensate this loss. The actual loss caused by the man's entering is probably only part of this maximum 0.3 per cent. Obviously, however, one should be careful not to use the entering device too often.

The Ventilating System

Rate of Ventilation.—It has been mentioned before (p. 20) that with a respiration apparatus involving gas analysis, a concentration of about 1 per cent CO_2 should be maintained in the outflowing air. In this case the error of the gas analysis, even if it is as high as 0.01 per cent of the gas analyzed, will not cause an error of more than 1 per cent of the result.

TABLE 5

Basal Metabolism and Optimal Rate of Ventilation

Weight of animal		Basal metabolism, H_b	Amount of CO_2 produced*	Optimal rate of ventilation	
				Basal	Four times basal
kg	*pounds*	*therms per 24 hours*	*liters per 24 hours*	*cubic meters per 24 hours*	*cubic meters per 24 hours*
100	220	2.27	378	38	152
200	440	3.83	638	64	256
300	660	5.13	855	85	340
400	880	6.44	1,073	107	428
500	1,110	7.61	1,268	127	508
600	1,320	8.72	1,453	145	580
700	1,540	9.81	1,635	163	652
800	1,760	10.82	1,803	180	720

* $\frac{H_b}{6.0} \times 1{,}000$.

In order to obtain this concentration of 1 per cent CO_2 in the outflowing air, the rate of ventilation should be 100 times as high as the rate of CO_2 production inside the chamber. This, of course, is true only for lengthy experiments in which the chamber volume is small compared with the volume of air passed. (See "Dimensions," p. 16.)

The results already obtained by several workers on basal metabolism in its relation to body size permit the estimation of the rate at which CO_2 is produced and hence of the necessary rate of ventilation for obtaining approximately 1 per cent CO_2 in the air leaving the chamber.

The basal metabolism of warm-blooded animals is 72 Cals. per $W^{\frac{3}{4}}$ in 24 hours where the weight W is expressed in kilograms (Kleiber, 1932, p. 317). Using this figure, and assuming that 1 liter of CO_2 corresponds to a heat production of 6.0 Cals.—an estimate which, for basal metabolism, is probably correct (Benedict and Ritzman, 1927, p. 147)—one obtains for the heat production, CO_2 production, and optimal rate of ventilation, the data given in table 5. The ventilation required for a heat production four times the basal is also given. This rate is probably the maximum to be considered.

The apparatus can easily be adjusted to very different rates of ventilation. The rate most often used is 200 cu. m a day, or 8 cu. m an hour, which corresponds to a maximal intensity (during the time when the cylindrical part of the pipette passes the surface of the water) of 3.8 liters a second.

Aspirator.—The aspirator consists of four large pipettes, two for each chamber, which are alternately immersed in water and withdrawn. During the upward movement, the water is flowing out of the pipette, and a corresponding amount of air is being sucked into it. During the downward movement, this air is pushed out by the entering water. A system of valves is operated so that the air is sucked from the chamber to the pipette and is pushed from the pipette to the outside. As the chamber itself is connected to the outside air by means of a pipe line (see "Air Duct," p. 29) outdoor air enters the chamber at the same time as air from the chamber is sucked into the aspirator pipette. There is thus a current of air from outside into the chamber, from the chamber to the aspirator, and from the aspirator to outside (fig. 1). This current of air is intermittent.

The method of pipetting the air has an advantage over the ordinary method of driving the air through a gas meter by a blower (Kühn *et al.*, 1894) or of using the gas meter itself as the aspirator (Rubner, see Wolpert, 1896; Krogh and Krogh, 1913). The error of the volume measured with these pipettes is negligible. Any difference in the water level of a wet gas meter, which is regarded as more accurate than a dry meter, introduces a considerable error. A difference of 1 cm in the water level in the water basin of our aspirator means a difference of only about one ten-thousandth of the total volume.

The gas meters in other apparatuses have to be calibrated from time to time. This calibration is generally carried out by means of a carefully constructed large cylinder, moved up and down in a water or oil seal for accurate measurements of air quantities. Our aspirator is itself an accurate calibrating device, as the volume of the pipette can be determined by weighing the amount of water that fills the pipette.

Compared with the Blackslees mercury pump, our aspirator has the advantage that the volume per period (the volume of the pipette) can be determined more directly and accurately than the volume per period of the mercury pumps. Möllgaard calibrates the mercury pumps with a gas meter (Möllgaard and Anderson, 1917, p. 76). A further advantage of our aspirator compared with the mercury pumps is that the air is measured over water, so that it is always saturated. In addition, the temperature of the aspirator changes but slowly, because of the large amount of water present and the resultant large heat capacity.

The form and dimensions of the air pipettes are shown in figure 5 (p. 21). The capacity of each of the four pipettes is 225 liters. The pipettes are made of 16-gauge sheet copper. The lower end is formed by a pipe 10 cm long and 7.6 cm wide.

For linear velocities of the aspirator pipettes over 1.5 cm per second, corresponding to intensities of the air current exceeding 4 liters per second, the lower outlet of the pipettes has been found too narrow. The friction of the water entering the pipette then interferes with a proper downward movement. The lower outlet could easily be made 15 cm wide without impairing the accuracy of the aspirator.

Just 5 cm from the lower end of the pipette is a 1.9 cm side outlet, *T*, where a screw cap may be removed and a rubber hose connected for calibration. Also, for the purpose of checking the volume of the pipette, the lower end of the 7.6-cm pipe is threaded so that one may close it with a screw plug during calibration.

The upper end of the pipette is connected to two concentrically fixed pipes of 5 cm and 10 cm inside diameter respectively. The inner tube is 135 cm long; the outer 125 cm. The latter is connected at the top to a collar 20 cm in diameter. The two pipes form a water seal for the fixed 7.6-cm pipe of the air duct (*AD*, fig. 5), along which the aspirator pipette slides up and down.

The collar at the top is to prevent large differences in the water level of the seal as a result of displacement by the fixed 7.6-cm pipe.

The basin for the water in which the four aspirator pipettes are moved up and down is 451 cm long, 90 cm wide, and 145 cm deep. These are the maximal inside measurements. The basin is built of reinforced concrete along a wall of the room in which the respiration apparatus is located. The free side wall is 7.6 cm thick and is reinforced by four vertical ribs. A footing 30 cm high and 25 cm wide surrounds the bottom of the basin on three sides. In planning the floor inside the basin, care has been taken to obtain enough slope for draining mercury that might be lost from sampling and collecting devices over the basin (fig. 6). The minimal grade, 26 per cent, has been obtained by making two outlets instead of only one. These outlets, which go through the floor of the room, are connected to the sewer by means of iron goosenecks, each provided with a shut-off valve. At the lowest part of the gooseneck is a small outlet valve for draining mercury. An overflow installed in one of the end walls of the basin maintains a definite water level.

Each aspirator pipette hangs on two chains. The weight of one pipette, including the water seal, is 85 kg.[6] Each chain, therefore, has to

[6] Of this weight, 25 kg is purposely added in the form of a strap of sheet lead fastened to the bottom of the pipette. This lead strap is not shown in the figure.

carry a load of 43 kg. The chains connect the pipettes by pairs, as shown in figure 6, and run on 19-tooth sprockets (fig. 6), which make about 6 r.p.m. An extra chain running on two 25-tooth sprockets connects the two pairs of pipettes so that the one motor moves all four pipettes. Two

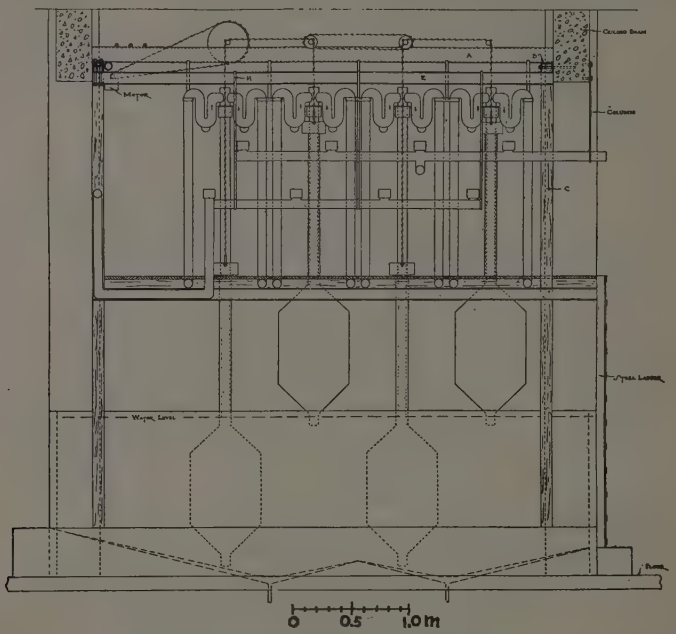


Fig. 6.—Aspirator section.

sprockets of 114 and 57 teeth, respectively, are fixed on to the first shaft (left of fig. 6; only one sprocket is drawn). Either of these sprockets may be connected to the driving machine by a chain.

The driving mechanism consists of a 1-hp. electric motor of 1,710 r.p.m. connected to a worm-gear unit that reduces the speed to 38 r.p.m. A gear connection to the drive shaft allows a further reduction of the speed, which, in our aspirator, is usually kept at 21 r.p.m.

A 25-tooth sprocket (connecting with the 57 or 114-tooth wheels) is mounted as a screw nut on a threaded part of the drive shaft between two clutches. This arrangement is made in order to disengage the drive of the aspirator pipettes when the motor reverses so that there is a pause before they are moved back. This is advantageous for the measurement of the air quantity in the pipettes, because the air has time to reach a

definite pressure between the closing and opening of the valves. Incidentally, the driving mechanism is not forced to reverse under load.

The length of time during which the pipettes remain in their end positions is regulated by two pins in the threaded part of the 21-r.p.m.

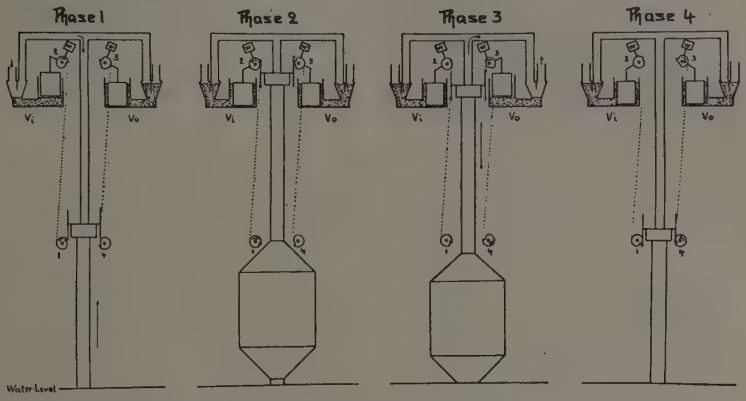


Fig. 7.—Operation of aspirator valves:

Phase 1: The aspirator pipette has just started to move upwards. The left spring hook at the head of the pipette has turned bushing No. 1, which through the chain has transmitted the motion to bushing No. 2, and thus opened the inlet valve *Vi*. The outlet valve is closed; air flows from the respiration chamber into the aspirator pipette.

Phase 2: The aspirator pipette has reached its upper end position; the left pushing rod at its head has closed the inlet valve *Vi* by turning bushing No. 2.

Phase 3: The motor has been reversed; the aspirator pipette has just started to move downward. The right-hand spring hook at its head has turned bushing No. 3 and opened the outlet valve *Vo*. Air flows from the pipette to outside.

Phase 4: The aspirator pipette has reached its lower end position; the right-hand pushing rod at its head has just turned bushing No. 4 and consequently by means of the chain at the right has closed the outlet valve *Vo*. The motor is reversed and phase 1 starts.

shaft at right angles to the shaft. Each of these pins drives the screw sprocket in one direction by engaging a horizontal extension of this sprocket. When the motor reverses, the shaft instead of turning the sprocket backwards will first disengage it, and by the action of the screw threads the sprocket is moved over to the other pin with which it engages, consequently transmitting the reverse movement to the pipettes. The distance between the two pins in the 21-r.p.m. shaft thus determines the length of time during which the pipettes are stopped at their end positions.

The valves in the air duct between the chamber and aspirator pipette, and between the latter and outdoors, are operated automatically by lifting up and lowering water in an air funnel with a central partition.

The valves, made of sheet copper, are shown schematically in figure

7. The connections for the air duct are above the funnel, the lower end of which is connected to a water container by means of a pipe 6 cm wide. The water container is a cylinder 17.8 cm in diameter and 30 cm in

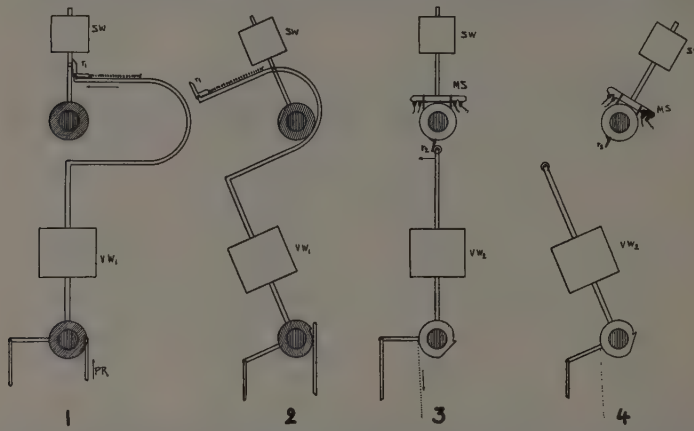


Fig. 8.—Reversing switch: *MS*, 4-pole mercury switch mounted on a bushing; *PR*, left-hand pushing rod at the head of aspirator pipette; r_1, ratchet with spring mounted on an extension of the weight rod of the inlet valve; r_2, ratchet mounted to same bushing that carries mercury switch; *SW*, weight (approximately 1 kg) mounted on a bushing on same shaft as that carrying the mercury switch; VW_1, weight approximately 2 kg) of mechanism controlling the inlet valve of the aspirator pipette; VW_2, weight (approximately 2 kg) of mechanism controlling the outlet valve of the aspirator pipette.

Phase 1: Aspirator pipette No. 4 is just moving toward its upper end position; the left-hand pushing rod *PR* at its head turns the bushing of the mechanism of the inlet valve and moves the weight VW_1 from right to left. The extension of the weight rod pushes the switch weight *SW* to the left and the upper bushing is turned on its shaft and transmits its motion to the bushing with the mercury switch.

Phase 2: By means of a pin in the upper shaft fitting into a slit in the upper bushing, this bushing when turning slides along the shaft so that after the weight *SW* has passed its highest position, it drives the bushing to its end position, and disengages the ratchet r_1.

Phase 3: Aspirator pipette is approaching its lower end position. The right-hand pushing rod at its head turns the bushing of the mechanism of the outlet valve by means of a chain, and moves the valve weight VW_2 to the left. The rod of this weight engages with ratchet r_2 and turns the bushing with the mercury switch *MS* and also that with the switch weight *SW* to the right.

Phase 4: After passing its highest position, the switch weight *SW* turns the bushing to its end position and disengages ratchet r_2.

height. In it is hung a cylinder 18 cm long, made of a piece of copper tubing 15.2 cm wide, both ends closed with sheet copper. This cylinder is movable up and down practically without friction and works as a plunger which, in the lower position, displaces the water, bringing its level high enough to close the air funnel.

Figure 7 shows four phases of the automatic operation of the aspira-

tor valves. In this sketch, water container and air funnel are turned in their relative position 90°, and the bushings of the valves are also turned 90° to make the sketch clearer. All upper bushings are actually on the same shaft; and the lower bushings, too, require but one shaft.

When the aspirator pipettes reach the end position, the motor must be reversed. For this purpose a four-pole mercury switch (*MS*, fig. 8) is

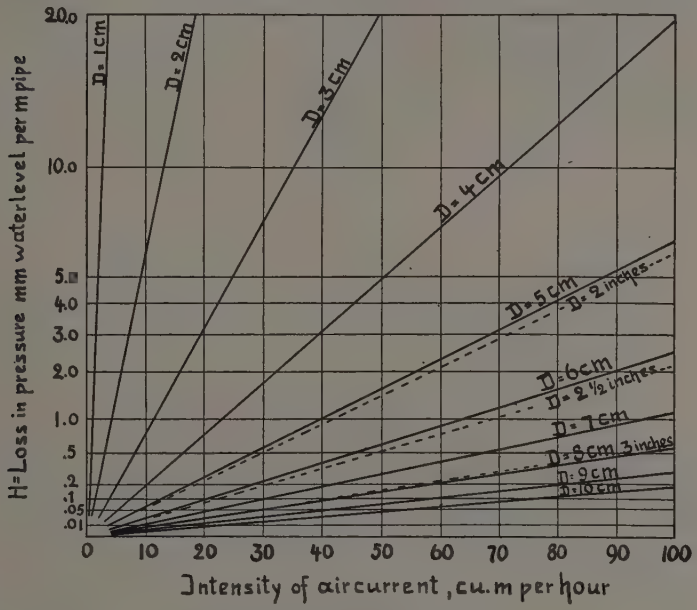


Fig. 9.—Resistance of air pipes.

mounted on a bushing that is turnable on a shaft. A second bushing on the same shaft turns the first one by an extending part, which fits into a groove of the first bushing. The second bushing carries a rod with a weight (*SW*, fig. 8) that secures a definite end position of the mercury switch.

Air Duct.—The air duct has a total length of approximately 23 m per chamber: that is, from the outside to the north chamber 5 m, from outside to the south chamber 7 m, from the north chamber to the distributor system 4 m, from the south chamber to the distributor system 2 m, from the distributor system to the pipettes 4 m, and from the pipettes to outside 10 m.

Galvanized iron pipes, with an inside diameter of 6.35 cm, have been chosen so that, even at a maximal velocity of the air, no considerable

difference in pressure should occur. For the construction of a respiration apparatus it is well to know the relations between the diameter of the pipes, the velocity of the air, and the resistance of the air duct.

These relations are given in figure 9, where the difference in pressure in millimeters water level per meter length of pipe is plotted against the intensity of the air flow in cubic meters per hour for pipes of different diameters from 1 to 10 cm. For a given pipe, the square root of the difference in pressure is proportional to the intensity of the air current.

The graph is based on the following formula of Pohle, used by gas engineers for estimating the diameters of gas pipes (Gregorovius, 1927, p. 712):

$$Q = \sqrt{\frac{D^5 H}{2sL}} \tag{20}$$

where

Q = intensity of air current in cubic meters per hour
D = diameter of air duct in cm
H = difference in pressure at the end of the duct in mm water level
s = relative density of the gas (air = 1.0)
L = length of air duct in meters.

According to this formula, the difference in pressure for 10 m of 6.35 cm pipe line at an intensity of the air current of 20 cu. m per hour should not exceed 1 mm water level. The difference actually observed in our aspirator is about 4 mm. As calculation shows, the largest part of this difference does not result from the resistance of the air duct as such, but occurs probably in the aspirator valves—a conclusion confirmed by actual observation with an outlet of the air duct near the valve open and closed.

A distributor system is so arranged in the air duct that each chamber can easily be connected to any one, or to two, or to all four of the aspirator pipettes by merely putting a U-shaped connecting tube from one oil seal to another. One of these changeable connection units is shown in figure 10.

A system of 2-inch downspouts with many holes inside the chamber causes the fresh air to enter the chamber simultaneously at different places and thus to mix with the chamber air quickly, so that at any given time the composition is uniform. A similar system of downspouts is used to take the air that passes from the chamber to the aspirator simultaneously from different parts of the chamber. This system combined with the fan, which rotates the air inside the chamber (see "Air

Conditioning System," p. 20), gives the air leaving the chamber at any time the average composition of the air inside.

Regulator for Air Pressure.—It is almost impossible to make large respiration chambers absolutely air tight. The leakage, however, does not affect the result of the experiment if, first, air leaks only into the

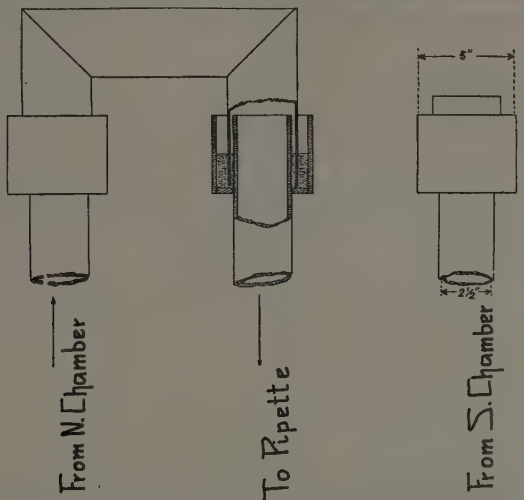


Fig. 10.—Air-duct connection. The central pipe leads to the aspirator pipette, the pipe to the left to the north chamber, the one to the right to the south chamber. The figure shows the connection made for the air flow from the north chamber to the aspirator pipette. The connection to the south chamber is closed.

chamber, not out, and if, second, the air leaking in has the same composition as the air outside the building, which is sucked into the chamber.

In order to prevent a leaking of air from the chamber to outside, a negative pressure is maintained inside. For this purpose a regulator is installed on each chamber. These regulators close the air ducts leading to the chambers as soon as the pressure inside is almost as high as that outside.

The air flowing from outside into the chamber has to pass through the funnel shown in section at the right side of figure 11. The funnel is 4 cm wide at the lower end and is 24 cm long (see plan). Dividing it lengthwise in two parts is a central partition, the lower end of which is 4 cm above the lower end of the funnel, as shown in the cross section. Oil contained in the oil box *BO* (fig. 11) seals the end of the funnel in the closed position; but, in the position of the regulator shown in the section, the oil leaves a passage open below the central partition.

The oil box swings on a frame to which it is fastened by the pins *b*, *b* (see plan and elevation). This frame is turnable on a horizontal knife-edge, *K*, and carries an inverted sheet-metal box, *BS*, the free ends of which are immersed in oil. The air inside this box mixes freely with the air in the chamber by an opening, *c*, through the chamber roof.

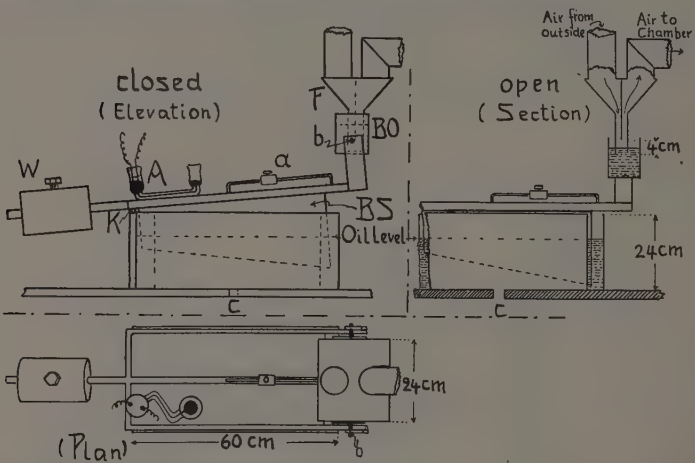


Fig. 11.—Regulator for air pressure: *A*, alarm device; *a*, adjusting weight for fine adjustment of the portion of the regulator; *b*, support for movable oil box; *BO*, sheet-metal box containing mineral oil; *BS*, inverted sheet-metal box fixed to the frame which also carries the box *BO*; *C*, opening in the roof of the respiration chamber connecting the chamber with the regulator; *F*, air funnel with a central partition conducting the air current from outside to the respiration chamber; *K*, knife edge on which the movable frame carrying the boxes *BO* and *BS* is suspended on a frame fixed to the roof of the respiration chamber; *W*, counter weight for approximate adjustment of the regulator.

A weight, *W*, of 20 kg on the left side of the frame and a small adjusting weight, *a*, above the air box, *BS*, can be so placed that the regulator is in the position shown in the elevation (fig. 11) when the air pressure in the air box (which is the same as that inside the respiration chamber) is equal to the air pressure outside. In this position the oil level in the oil box, *BO*, is above the lower end of the central partition of the air funnel, and the air duct is closed. If by the action of the aspirator the pressure in the chamber and, consequently, also in the air box, *BS*, becomes lower than the outside air pressure, the air box is pressed down by the higher outside pressure. Consequently, the regulator reaches the position shown in the section (fig. 11), and the air duct is open. Because the difference in pressure acts upon a large area, the regulator is very sensitive: it opens at a difference in pressure of 2-mm water level.

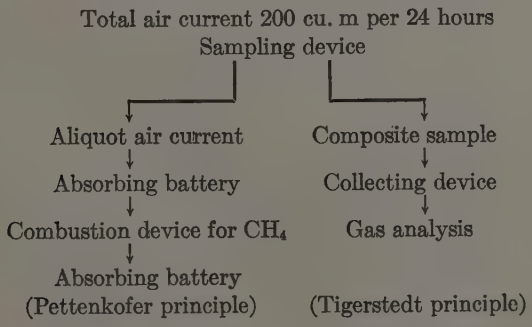
Air-Analyzing System

General Scheme.—As has already been mentioned, the California apparatus is a combination of the Pettenkofer and Tigerstedt systems. In Pettenkofer's system a small part of the air leaving the chamber (an aliquot air current) is driven over an absorbing device for CO_2. The absorber may be $Ba(OH)_2$ solution in which the amount of CO_2 absorbed can be determined by titration; or the CO_2 in the aliquot air current may be absorbed by soda lime, Ascarite, or a similar absorber, and determined gravimetrically. Instead of absorbing the CO_2 in an aliquot air current, Tigerstedt uses the analysis of a composite gas sample—that is, one obtained by collecting small portions of the outflowing air at regular intervals. The Pettenkofer system can be developed so that it works very accurately, as proved by the tests made by Kellner and Köhler (1900). The improvement obtained by Tigerstedt's principle is mainly the possibility of measuring O_2 consumption as well as the production of CO_2.

The advantage of using both methods simultaneously is that the results on CO_2 obtained by the two methods serve as checks for each other. The two principles of measuring the respiratory exchange have already been combined by Möllgaard (1929, p. 68).

Both results depend upon the measurement of the ventilation; and, even if a mistake is made in measuring the air quantity—for example, a wrong calibration of the aspirator—the result of the Pettenkofer method may agree with that of the Tigerstedt method. Nevertheless, there are numerous other sources of error in the respiration trial which may be detected by applying the two methods simultaneously, so that the advantage of combining them is beyond doubt.

The following scheme gives the general outline of the double analyzing system:



Air-Sampling Device.—The air-sampling device consists of four pipettes: a 1-cc and a 20-cc pipette for each of the two respiration chambers. Mercury is moved up and down in these pipettes simultaneously with the movement of the aspirator; and, in consequence, air is sucked

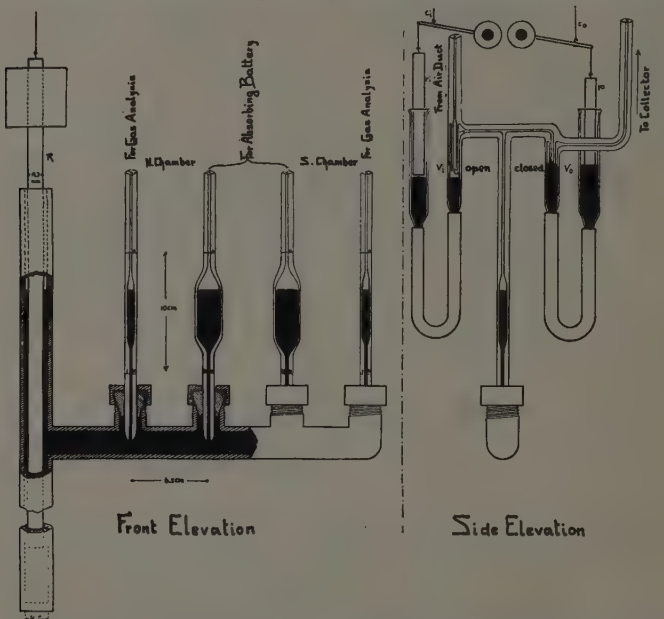


Fig. 12.—Air-sampling device: C_i, connection to mechanism of inlet valve of aspirator pipette No. 1; C_o, connection to mechanism of outlet valve of aspirator pipette No. 1; R, ½-inch steel rod with approximately ½ kg extra weight; a chain connects this rod to a transmission driven by aspirator pipette No. 1; r_i, steel rod shown in its upper position leaving the inlet valve Vi of the sampling device open; r_o, similar steel rod shown in its lower position, closing the outlet valve Vo.

from the air duct into the sampling pipettes and from there is pushed to the collecting device (1-cc sample) or to the absorbing battery (20-cc sample).

By means of rubber stoppers the four pipettes are fixed in ¾-inch diameter short nipples, as shown in the front elevation of figure 12. The stoppers are pressed tight by a screw cap. In this way is obtained a mercury-tight connection, flexible enough to avoid strains on the glass. The lower ends of the pipettes extend into the mercury. Impurities tend to remain at the surface, and the mercury inside the pipettes may still be clean when that in the connecting pipes is contaminated. This, of course,

is true only for those contaminating substances that do not amalgamate. Figure 12 is slightly schematic, the horizontal part of the mercury container being actually made up of tees and short nipples. This horizontal part is connected to a vertical ¾-inch iron pipe[7] 47 cm long (fig. 12). A steel rod *R*, 12.7 mm in diameter with a 1-kg weight at the top, is hung to a transmission so that it moves up and down simultaneously with one aspirator pipette but with only half the linear velocity of the pipette.

This movement of the steel rod in the vertical pipe causes a corresponding movement of the mercury level in the sampling device. The total difference in level of the mercury is 10 cm.

The top of each sampling pipette is connected to an inlet valve *Vi* and an outlet valve *Vo*, whose construction is shown in the side elevation of figure 12. As the pipettes are made of Pyrex glass, the blowing of the double seal offers no difficulty. The lower end of each valve is connected, by means of rubber tubing, to a glass tube of 13-mm inside diameter, the position of which can be varied for adjusting the level of the mercury contained in the valve. The mercury level is raised and lowered for closing or opening the valve by a steel rod, *ri* or *ro*, 10 mm in diameter, movable inside the glass tube and hung to a lever that is fixed to a shaft. On the left side, this shaft is connected to the inlet valve of the aspirator pipette No. 1 by the connection *Ci;* on the right side, by the connection *Co* to the outlet valve of the same aspirator pipette.

If the aspirator pipette is moving upward and opening its inlet valve as described on page 27, figure 7, the inlet valves of the sampling pipettes, *Vi*, are opened simultaneously by means of connection *Ci*, which lifts the steel rod *ri* to the position shown in figure 12.

The steel rod *R* of the sampling device is lifted by the upward movement of the aspirator pipette; consequently the mercury level in the sampling pipettes is lowered, and air from the air duct is sucked into them. When the aspirator pipette reaches its upper end position, its inlet valve is closed, the steel rod *ri* is lowered, and, in consequence, the sampler valve *Vi* closed. Now 1 cc and 20 cc respectively of air are enclosed in the sampler pipettes as samples of the 200 liters of air contained at the same time in the aspirator pipette. When the aspirator pipette begins to move downward, opening its outlet valve, the outlet valves of the sampling device are opened simultaneously. The steel rod *R* is lowered into the mercury, which rises and pushes the air samples out from the sampler pipettes to the collecting device (1-cc sample) and to the absorbing battery (20-cc sample). Originally the air in the sampling de-

[7] Neither galvanized nor brass pipes or fittings can be used, as they would contaminate the mercury.

vice had the same humidity as that in the chambers. The moisture in the air in connection with other constituents, particularly H_2S, was probably responsible for a rather quick contamination of the mercury. Re-

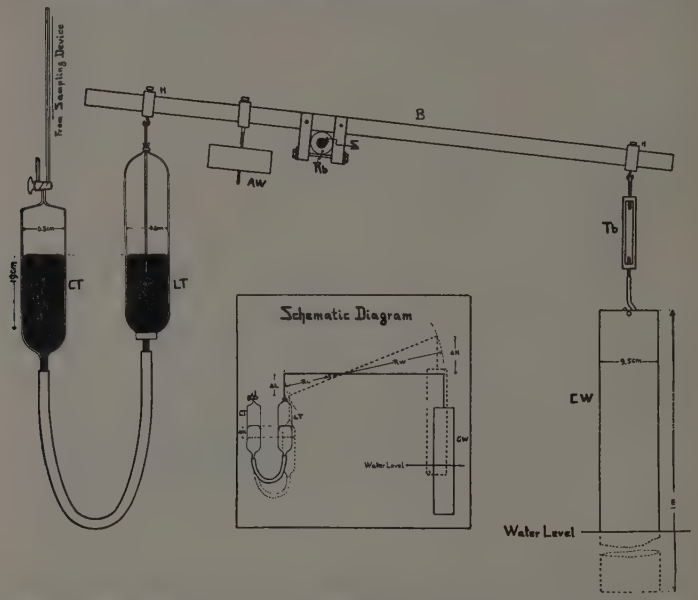


Fig. 13.—Air-collecting device: α, angle between two directions of beam of balance; *AW*, adjusting weight; *B*, beam of balance; *CT*, air-collecting tube with capillary connection to air-sampling device; *CW*, counter weight, a copper cylinder partly immersed in water; ΔH, vertical movement of copper cylinder; ΔL, vertical movement of leveling bulb; *M*, difference in level of mercury in collecting tube; *H*, *H*, hangers for leveling tube and counter weight respectively; *LT*, leveling tube; *Rb*, roller bearing holding beam of balance on shaft; *RL*, lever arm for leveling tube; *RW*, lever arm for counter weight; *S*, steel shaft; *Tb*, turnbuckle for adjustment of counter weight.

cently the air sample has been drawn over CaCl in order to dry it. Since the introduction of this procedure the mercury remains clean.

Air-Collecting Device.—The 1-cc air samples regularly taken from the air duct are collected in glass containers over mercury. Not more than 1-cm mercury-level difference in pressure should occur during collection, because a larger difference would tend to upset the working of the sampling device.

The problem has been solved by connecting the fixed collecting tube *CT* (see schematic diagram in fig. 13) to a leveling tube *LT* hanging from one arm of a balance. The top of the leveling tube is open.

Mercury driven from the collecting tube by the air sample enters the leveling tube and increases its weight. The leveling tube is lowered until its increase in weight is compensated by the increased weight of the counter balance, resulting from the loss of water displacement. The dimensions are so chosen that the equilibrium is established when the mercury in the leveling tube is at the same level as that in the collecting tube—that is, when the air sample in the collecting tube has the same pressure as the outside air.

If the gas pressure in the collecting tube is equal to the barometric pressure, the collecting device is in equilibrium at any position. For the necessary relation of the various dimensions, the following equation has been developed:

$$F_w = \left[\frac{F_c}{1+\frac{F_c}{F_l}} \times 13.55 - \frac{q}{2}\right]\left(\frac{R_l}{R_w}\right)^2 \qquad (21)$$

where

F_w = cross-section area of the counter weight in the water
F_c = cross-section area of the mercury cylinder in the collecting tube
F_l = cross-section area of the mercury cylinder in the leveling tube
q = weight per cm of the connecting rubber tube filled with mercury
R_l = radius of the arc described by the hanger on the beam for the leveling tube
R_w = radius of the arc described by the hanger on the beam for the counter weight.

If the leveling and the collecting cylinder have the same diameter, $F_c = F_l$ and if, for an approximation, q may be neglected, the relation may be simplified as

$$\frac{R_l}{R_w} = \frac{D_w}{D_m}\sqrt{\frac{2}{13.55}} \qquad (22)$$

where D_w is the diameter of the counter-weight cylinder in the water and D_m the diameter of the mercury in the leveling and the collecting cylinders.

The special construction of the collecting device is shown in figure 13. The collecting tube CT and the leveling tube LT are 7 cm in outside diameter. The cylindrical part of the collecting tube is 19 cm long; the leveling tube, 1 cm longer. The top of the collecting tube is a cone, made as flat as possible because the collecting device is working correctly only

when the mercury level is in the cylindrical part of the collecting tube.

The beam, *B*, to which the leveling tube is hung by means of the adjustable hanger, *H*, is a 1.27 × 2.54 cm steel rod fixed to a 2.54-cm-long roller bearing on a 19-mm steel shaft, *S*.

The counter weight, *CW*, is a 100-cm copper pipe of 9.5 cm outside diameter (12 gauge). To the lower end is soldered a copper plate; the upper end is hung to the beam by means of a turnbuckle, *Tb*, which permits the adjustment of the weight by lowering or raising the copper tube in the water and thus varying its water displacement.

An adjusting weight, *AW*, is installed in order to bring the center of gravity of the beam into the center of the shaft upon which it is balanced. For this purpose the additional weight may be moved horizontally along the beam as well as perpendicularly to its direction.

Four collecting units, as described, are installed on the same shaft, *S*. A capillary three-way stopcock is connected to the top of the collecting tube. The capillary on the left is for taking the sample out of the collecting tube for gas analysis. The capillary on the right side leads to a distributor system consisting of two three-way stopcocks, so fixed together by capillaries that the outlet of the 1-cc sampling pipette may be connected to either of two collecting tubes. Thus, for starting a new experimental period, only two stopcocks have to be turned. By turning a stopcock, one may also connect the collecting bulbs to the outlets of the 20-cc sampling pipettes for taking composite samples during an experiment of short duration—for example, one hour.

The collector units are located at the same end of the water basin as the sampling device.

Gas Analysis.—The gas samples are analyzed in two apparatuses, one for CO_2 and one for the sum of CO_2 and O_2. These apparatuses and their operation are described in a separate paper (Kleiber, 1933*a*).

Absorbing Battery and Titrimetric Determination of CO_2.—The determination of the CO_2 in the aliquot air current is made by titration, which has the advantage over the gravimetric method of being more specific for CO_2 than is the increase in weight of the absorbing system: any substance will increase the weight, but only substances with acid or basic properties will influence the titration. It was decided, therefore, to use the old Pettenkofer principle of absorbing the CO_2 in a $Ba(OH)_2$ solution and determining the absorbed amount by titration.

The direct titration of CO_3^{--} is subject to a considerable error (Kolthoff and Menzel, 1928, p. 126). The influence of hydrolysis, which causes the relatively large error of titration, may be reduced by maintaining a low concentration of carbonate ions. For that purpose $BaCl_2$ is added to the solution in order to insure at all times an excess of Ba^{++}. This pro-

cedure is known as Winkler's method of titrating alkali in the presence of carbonates (Kolthoff and Menzel, 1928, p. 32).

The influence of $BaCl_2$ upon the accuracy of the titration in the presence of carbonate is shown in the neutralization curves in figure 14 for

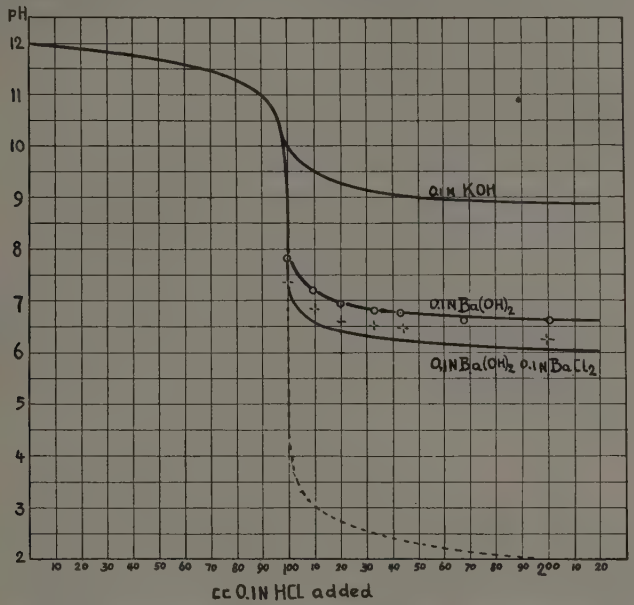


Fig. 14.—Titration curve of KOH and $Ba(OH)_2$ solution containing carbonates with HCl.

the titration with 0.1 *N* HCl of 1 liter 0.1 *N* alkali after absorption of 0.045 mols CO_2. If a barium electrolyte is present, the concentration of carbonate ions may be calculated from the solubility product of $BaCO_3$ and the concentration of the Ba ions. The concentration of HCO_3^- is a function of the concentration of CO_3^{--} and H^+ ions; and, further, the concentration of H_2CO_3 is a function of the HCO_3^- concentration:

$$(CO_3^{--}) = \frac{S}{(Ba^{++})} \tag{23}$$

where S = solubility product of barium carbonate $= 7 \times 10^{-9}$.

$$(HCO_3^-) = \frac{K_w(CO_3^{--})}{Ka_1(OH^-)} \tag{24}$$

where K_w = ionic product of water $= 10^{-14}$ and
Ka_1 = first dissociation constant of carbonic acid $= 3 \times 10^{-7}$.

$$(H_2CO_3) = \frac{K_w(HCO_3^-)}{Ka_2(OH^-)} = \frac{K_w^2(CO_3^{--})}{Ka_1 \times Ka_2(OH^-)^2} \quad (25)$$

where Ka_2 = second dissociation constant of carbonic acid = 6×10^{-11}. The concentration of barium ions has been assumed to remain constant; that is, the difference in degree of dissociation between $Ba(OH)_2$ and $BaCl_2$ has been neglected, as well as the number of mols of Ba precipitated as carbonate.

In the case of KOH as absorber, the concentration of CO_3^{--} has been calculated by considering that the total quantity of CO_3^{--} (including the amount of HCO_3^- and H_2CO_3) is constant (namely, 4.5×10^{-2} mols) and that HCO_3^- and H_2CO_3 may be expressed as functions of CO_3^{--}. It has been assumed that K_2CO_3 is completely dissociated. The difference in the volume of the solution by titration has also been neglected.

The amount of HCl necessary for changing the pH of the solution for one unit is the sum of the amount necessary for this change at ordinary neutralization (that is, without hydrolysis) plus the amount required to produce the increase of HCO_3^- and H_2CO_3 for that change of pH caused by hydrolysis.

The equations

$$CO_3^{--} + H_2O = HCO_3^- + OH^- \quad (26)$$

and

$$HCO_3^- + H_2O = H_2CO_3 + OH^- \quad (27)$$

show that for each mol of HCO_3^- formed by hydrolysis one equivalent of H^+ is required to neutralize the $(OH)^-$ produced and that for each mol of H_2CO_3 formed from HCO_3^- an additional equivalent of H^+ is required.

The curve for the KOH solution (fig. 14) shows that the buffering influence of the K_2CO_3 present prevents a sharp end-point of titration and thus causes a larger error. The curve with $Ba(OH)_2$ indicates the superiority of this absorbent over KOH, producing a more rapid change of pH at the neutralization point. The addition of $BaCl_2$ increases the fitness of the solution for titration only slightly, as is shown by comparison of the curve for 0.1 *N* $Ba(OH)_2$ and 0.1 *N* $BaCl_2$ with the one for 0.1 *N* $Ba(OH)_2$ alone. The curve for neutralization without carbonate, shown as a dotted line, is added for comparison. The circles on the graph in figure 14 show the data for the titration of 0.1 *N* $Ba(OH)_2$ alone with 0.1 *N* HCl actually measured by potentiometric determination with the quinhydron electrode. The agreement between the calculated curve and the result of the measurement is very good.

The crosses on figure 14 mark the results obtained by direct measurement for the titration of the 0.1 *N* $Ba(OH)_2$ solution containing 0.1

N $BaCl_2$. The value of the calculated curve for this titration is lower than measured data—a discrepancy that can be explained by the escaping of CO_2 from the solution during the titration, since the formation of gas bubbles when acid is added has actually been observed.

It has been found practical to use for absorption 0.2 *N* $Ba(OH)_2$ solution containing 0.2 *N* $BaCl_2$, titrated with 0.2 *N* HCl so that 1 cc of titrated solution corresponds to about 2 cc of CO_2 gas. Two-tenths per cent alcoholic phenolphthalein solution is a satisfactory indicator. Recently a mixed indicator of the following composition, suggested by Kolthoff and Menzel (1929, p. 65), has been used:

1 part $\frac{1}{10}$ per cent cresol red sodium in water
3 parts $\frac{1}{10}$ per cent thymol blue sodium in water.

The main advantage of this indicator over phenolphthalein for this purpose is its solubility in water, which permits its addition to the stock solution of $Ba(OH)_2$ and also to the HCl solution used for titration (10 cc of the indicator solution per liter). This procedure guarantees a constant concentration of the indicator throughout the titration. It was thought inadvisable to add the alcoholic solution of phenolphthalein, for fear the alcohol might evaporate, be oxidized in the combustion tube, and falsify the result of the CH_4 determination.

In designing the absorbing battery, the aim was to obtain a complete absorption of the CO_2 with no resistance to the passing air, because such a resistance would cause a back pressure that would disturb the sampling device if higher than 1 cm mercury level. For this reason the method of bubbling the gas through the absorbing liquid (Pettenkofer, 1862) has been abandoned.

The velocity of absorption of CO_2 from the air by a liquid is assumed to be limited mainly by the rate of diffusion in the liquid phase. The surface of the liquid tends to become saturated with CO_3^{--}, and more CO_2 can be absorbed only as new alkali diffuses to the surface. With $Ba(OH)_2$ as the absorbing fluid, a regular skin of $BaCO_3$ is formed, preventing or at least slowing down considerably the further absorption of CO_2. This phenomenon may be observed even in bubbling the gas through the liquid, for the white skin of $BaCO_3$ forms around every air bubble, making it questionable whether the great length of the Pettenkofer burette is really important for an efficient absorption of CO_2. If the limiting factor for the rate of absorption is the composition of the liquid phase at the surface, then in order to increase the efficiency of absorption one should maintain the absorbing power of the surface rather than increase its area. The carbonates must be replaced by $Ba(OH)_2$ faster than would take place by ordinary diffusion.

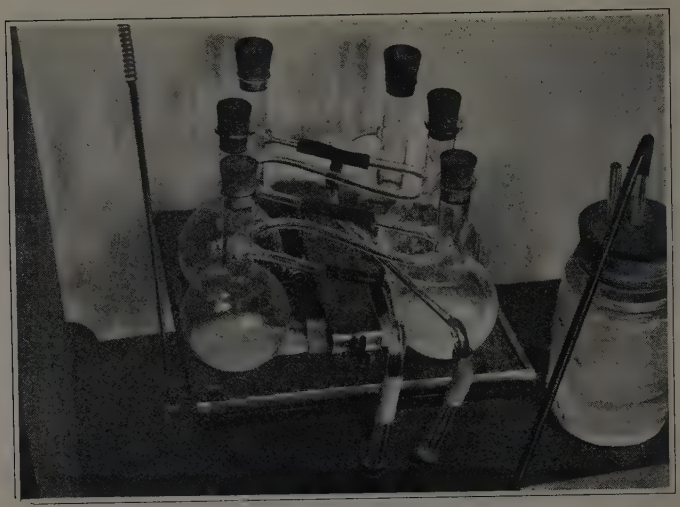


Fig. 15.—Absorbing battery.

Fig. 16.—Absorbing system and air combustion: *AL*, airlocks between first absorbers and combustion tubes; *CN*, *CS*, capillaries in which the aliquot air current flows from the sampling device to the absorber for north and south chambers respectively; *Na*, *Sa*, absorbers for CO_2 in the aliquot air current before air combustion for north and south chambers respectively; *Np*, *Sp*, absorbers for CO_2 in the aliquot air current after air combustion for north and south chambers respectively. The arrows indicate the directions of the aliquot air current.

An attempt was made to produce a shower of the absorbent through the gas in a fixed jar by means of a stirrer device. This device was, however, too complicated to handle; and, after a means of making a satisfactory flexible connection for the air of the aliquot air current had been found, the entire battery was moved instead of the stirrer only.

The absorbing battery now used consists of two pairs of 125-cc bottles and one pair of 25-cc bottles (fig. 15). Each two of the 125-cc bottles are connected by a 17-mm (outside diameter) glass tube at the bottom and a 5-mm glass tube between the necks. The 25-cc bottles have only the connection at the bottom. The three pairs of bottles are connected as shown in figure 15 and are mounted on a monel sheet 20×20 cm. The battery fixed on this sheet can be slipped on a cradle, as shown in figures

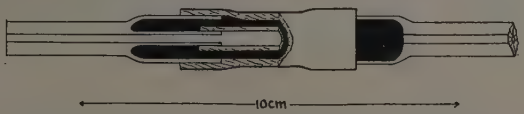


Fig. 17.—Flexible air connection for the conduction of the aliquot air current from the stable capillary duct to the rocking absorbing battery.

15 and 16. The latter figure shows how each cradle is connected to a camshaft, operated by a $\frac{1}{20}$-hp. electric motor with a reducing worm and gear so that it makes 6 r.p.m.

The flexible connection from the air duct to the rocking battery is made of two concentric rubber tubes (sulfur-free pure gum) with mercury between them. The inside rubber tube is thus surrounded by a tube of mercury which, though flexible, is not permeable to CO_2, as is the rubber tube (fig. 17). The amount of absorbent [0.2 *N* $Ba(OH)_2$ and 0.2 *N* $BaCl_2$] placed in the bottles depends upon the intensity of CO_2 production expected. Ordinarily 60 cc is measured in the first, 25 cc in the second, and 5 cc in the last pair. The air can pass freely from the neck of one bottle to that of the other (fig. 15) except in the last pair, where it must pass through the liquid in the connecting tube at the bottom in order to insure sufficient contact between the air and the liquid to remove the last trace of CO_2.

The difference in the efficiency of absorption when the battery is rocking and when it is not is shown by an experiment in which there was allowed to pass each minute 10 cc of air containing 1 per cent of CO_2 at a temperature of 18° C; 0.2 *N* KOH solution was used as absorbent in order to avoid the effect of the crust of $BaCO_3$ formed when $Ba(OH)_2$ is used. The result is given in table 6.

In this experiment only 33 per cent of the alkali in the first pair of bottles was used. The high efficiency of absorption, however, is main-

tained for a much larger amount of absorbed CO_2, as demonstrated by a respiration experiment with heifers where, through an error, only 50 cc of $Ba(OH)_2$ solution instead of 60 cc had been measured into the

TABLE 6

Efficiency of CO_2 Absorption in Battery: Rocking vs. Nonrocking

Pair of bottles	CO_2 absorbed			
	Cubic centimeters		Per cent of total	
	Nonrocking	Rocking	Nonrocking	Rocking
1	36.5	47.5	74	96
2	8.7	1.8	18	4
3	3.1	0.0	6	0
Total	48.3	49.3	98	100

first pair of bottles. This amount was used up to 89 per cent, and the absorption was still very efficient. The result is shown in table 7.

This absorbing system has a very small absorbing surface compared with the absorption by bubbling through a liquid or with a battery of

TABLE 7

Efficiency and Capacity for CO_2 Absorption

Pair of bottles	Absorbent, in cc *N*/5	Back titration, in cc *N*/5	Acid absorbed, in cc *N*/5	Absorbed, in per cent of total
1	52.9	5.7	47.2	97
2	26.5	25.2	1.3	3
3	10.6	10.5	0.1	0

soda lime or Ascarite. The air can pass freely from the neck of the first absorbing bottle to the neck of the fourth, and yet only a small part of the CO_2 molecules escape absorption in the first pair of absorbers. This result seems to confirm the preliminary assumption that the factor limiting the rate of absorption is the composition of the absorbing surface rather than the chance of the CO_2 molecules to hit the surface by the Brownian movement. By the action of the cradle, convection is added to the diffusion in the gas phase also, a circumstance which tends to increase the efficiency.

To keep the experiment continuous, two batteries are installed for each chamber so that one is absorbing while the other is slipped off the cradle for titration.

Air Combustion.—The amounts of combustible gases given off by ruminants are so large that the loss of energy through this process is to be considered. Of these combustible gases, CH_4 is the most important. Its production varies according to the amount and composition of the food and according to various other conditions not completely known.

The most pronounced influence on CH_4 production seems to come from carbohydrates, so that Kellner, as a result of special studies, gives certain relations between the amount of carbohydrates fed and the amount of CH_4 produced (100 grams of starch yielded 3.17 grams, 100 grams of saccharose 2.84 grams, of CH_4). The loss of energy for the production of CH_4 is given by Kellner as 10 per cent of the energy in the carbohydrates fed (Kellner, 1919, p. 94).

The relative importance of the CH_4 production in ruminants and the fact that this production is varied by factors not thoroughly known make the direct determination of this gas advisable.

Kühn *et al.* (1894) determined the CH_4 given off by the animals by passing an aliquot air current through a combustion tube heated by Bunsen flames placed underneath. The same scheme is followed in this apparatus; but the combustion tubes in the California apparatus are heated, not by Bunsen flames from outside, but by an electric current passed through a coil of nichrome wire inside them. The correct temperature (light red glow) is obtained in this apparatus with a power of approximately 400 watts. The hot wire itself seems to act as a catalyst for the combustion. Kellner obtained a complete combustion only by using platinum finely dispersed in kaolin as a catalyst (Kellner and Köhler, 1900), whereas in the trials here the result was the same whether or not the tube contained platinum asbestos. The combustion tube is made of silica 122 cm in length and 1.9 cm in inside diameter. The tube is surrounded by a nickel silver sheet as a reflector and is wrapped in layers of asbestos; the whole is placed inside a sheet-metal tube 10 cm. in diameter (fig. 16). A hole is made in the side of the cylinder for observing the glow. A piece of copper tubing, fixed to each end of the silica tube by means of a rubber stopper, conducts both the electric current and the aliquot air current to the heating coil. Small copper containers, soldered to the copper tubing, are filled with water to keep the copper tubing and, consequently, the rubber stoppers cool.

Three of these combustion units are installed as in figure 16. Two are continually in use during an experiment, while one is kept in reserve. Any of the combustion devices may be connected to the capillary duct of the aliquot air current of either the north or the south chamber so that the air, after having passed the absorbing battery that removes the CO_2, enters the tube where the CH_4 is oxidized to CO_2 and H_2O. The

CO_2 thus formed by the combustion of CH_4 is absorbed in a second set of rocking absorbing batteries through which the air passes after leaving the combustion tube. Between the first battery and the combustion tube, as well as at the end of the second battery, are air locks. The air of the aliquot current has to bubble through a column of water 1 to 2 mm in height, which is sufficient to prevent a flowing backward of the air, and not so high that the back pressure disturbs the sampling device. The bubbling through these air locks serves also as an indicator for the proper functioning of the aliquot air current.

Accessories

Device for Recording the Position of the Cow.—In energy-metabolism studies, it is interesting to know how much time the animal has spent in standing and lying, respectively. In order to get a record of her position, each cow has around the breast a belt, fixed to a cord that causes a mercury switch to be closed when she is lying and opened when she is standing. A roller from a typewriter is connected to a $\frac{1}{500}$-hp. electric motor by means of a speed reducer so that a point of its surface has a velocity of about $\frac{1}{2}$ mm a minute. A strip of adding-machine paper is moved with this velocity over the typewriter roll. On this paper glide two grease pencils, making a mark when the electric current mentioned above is open—that is, when the cows are standing. If a cow lies down, closing the mercury switch, the electric current passes a solenoid, which, in drawing in its core, lifts up the grease pencil. Thus a red mark appears on the paper for the time the cow was standing, forming a record when and how long she stood and how often she changed her position.

Motility Recorder.—In order to be informed about the behavior of the cow, one should know not only how long she has been standing, but also whether during that time she stood quiet or was moving around.

In order to have a record of this motility, the platform upon which the cow stands or lies is laid on four automobile inner tubes, size 34.0 × 5.0 in., filled with air to a pressure of approximately $\frac{1}{10}$ atmosphere. Whenever the cow moves (that is, displaces its center of gravity horizontally), any point of the platform, especially the corners, will move in a vertical direction because one tube will be more and another less loaded. One corner of the platform is connected to a lever that transmits the movement to a chain, which, in turn, operates a ratchet device so that the movements influence a productimeter in one direction only, causing their summation to be recorded. The difference in the readings of the productimeter gives an arbitrary measure for determining the motility of the cow during the corresponding time. This arrangement has been found more satisfactory than the originally planned combination of hinges and springs shown in figure 3.

Safety and Alarm Devices.—If, for any reason, the reversing switch (see p. 28) failed to work, the machine would force the aspirator pipettes beyond their end position; and, as the motor is strong (1 hp.) and the ratio of transmission very high (850:1 for the ordinary rate of ventilation), something would have to break before the overload switch of the motor would be thrown out. To safeguard against such a remote possibility, there is fixed above every aspirator pipette a switch that will be opened when the pipette goes 1 cm too high. These four switches are all in series with the push-button station for starting the motor, so that

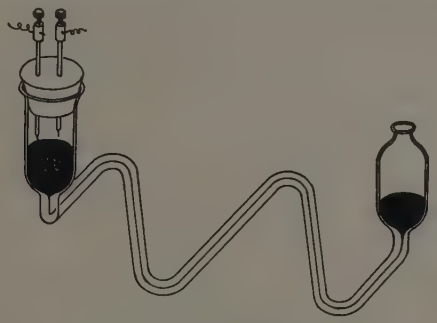


Fig. 18.—Alarm device.

if any of them is opened the electromagnetic switch is opened and, consequently, the electric power for the motor is shut off.

To prevent overheating, there is installed in each of the chambers, a bimetal thermostat control, which closes a direct current when there is a rise of 4° C above the desired temperature, and thus operates an electric alarm device, a line of which is connected to the room where the attendant on night duty sleeps.

The main safety device makes an alarm whenever there is no suction inside the respiration chamber. This condition may occur if the electric power is interrupted or if the machine is otherwise disturbed. It may occur when a valve of the aspirator does not work properly or when the chamber is leaking. The instrument that indicates this condition consists of two glass tubes 2 cm wide and 5 cm long, connected by a 1-mm capillary 50 cm long, bent in zigzag, so that the apparatus stands by itself (fig. 18). In the tube at the left are two platinum-pointed contact rods, connected to an electric battery and to an alarm bell which rings as soon as the mercury in the left bulb rises high enough to make contact between the two platinum points.

This apparatus is fixed to the top of the regulator for air pressure. If the air pressure inside the chamber equals that outside, the top of the

regulator is inclined (fig. 11).[8] In this position the mercury in the alarm device will flow from the right-hand bulb to the left. When the aspirator is working, causing a suction in the chamber, the regulator top is forced down to the horizontal position, allowing the mercury in the alarm device to flow from left to right. The position of the contacts in the left bulb is so adjusted that contact is made when the pressure inside the chamber remains equal to the outside air pressure for 1 minute (that is, the time required for the movement of an aspirator pipette in one direction).

At high experimental temperatures, for example, 30° C, a rise of the humidity to near saturation would be fatal for the cows. As a safeguard against such a rise in humidity, a small balance (*A* in fig. 4, p. 21) is fastened to the outlet of the brine pipe in the brine tank. One arm of that balance forms a small shovel; the other carries a mercury switch. The weights are so chosen that when no brine flows, the shovel closes the outlet of the brine pipe; and in this position the mercury switch is closed, operating the alarm system. When the brine flows, the shovel is forced down and the mercury switch is opened.

Device for Absorption of Ammonia in the Air of the Chamber.—In order to recover the NH_3 that is produced inside the chamber, a box lined with lead is installed at the front wall of each chamber (*N* in fig. 4). The box measures $61 \times 15 \times 10$ cm, inside measurements. In it a glass cylinder 11 cm in diameter and 53 cm in length is suspended by means of short pieces of glass tubing 1 cm in diameter, sealed to the ends of the cylinder. One of these pieces of glass pipe is connected to the shaft of a $\frac{1}{20}$-hp. electric motor with a speed reducer by means of a piece of rubber tubing so that the cylinder is turning at the rate of 86 r.p.m. Five liters of diluted $\left(\frac{N}{1}\right)$ H_2SO_4 are put into the box. By adding distilled water to compensate for the loss by evaporation, the level of the liquid in the box is maintained high enough during the experiment to keep the rotating glass cylinder wet. This procedure insures a good absorption of the NH_3 out of the air, which is driven through the condenser channel over the absorber by a fan (fig. 4).

Operation of Respiration Apparatus

The operation of the respiration apparatus may be described by giving the rules for an ordinary trial.

Two animals are so selected that they are as similar as possible in weight, age, development, and breed. Before the experiment starts, they are given the fixed amount of the experimental food daily for a period

[8] The tangent of the angle between the top and the horizontal is approximately 0.1.

of at least two weeks and are brought into the respiration chamber several times if they are not already acquainted with the apparatus. After the two weeks of preliminary feeding, and one or two mornings before the experiment begins, the animals are placed in the chambers. The evening before the beginning of the experiment, the chambers are closed and the machine is started, so that by the beginning of the experiment, the air in the chambers, in the air duct, in the capillaries of the sampling and collecting devices of the aliquot air current, and in the combustion tubes, has a composition similar to that on any morning during the experiment. Thus an error caused by the dead space in these devices is avoided.

The procedure for the beginning is as follows:

7:43 a.m. During the upward movement of aspirator pipette No. 1, draw small amounts of air into the sampling bulbs that have been connected to the air ducts for the momentary samples. Let these air samples out again, and repeat this operation in order to bring air of the correct composition into all connections. Record the figure of the aspirator productimeter at the end of this movement of No. 1 pipette. (See table 12 in Appendix.)

7:44 a.m. During the downward movement of aspirator pipette No. 1, take air from the air ducts into the sampling bulbs as the *momentary samples.*

7:45 a.m. First, during the end position and upward movement of aspirator pipette No. 1, close the collecting tubes that contain the composite air sample from the preceding day by turning two stopcocks for each tube.

Second, connect the other collecting tubes (which are full of mercury) to the sampling device by turning two stopcocks for each tube.

Third, connect the duct for the aliquot air current to the batteries, which have been filled with fresh alkali solution, by turning each of the six stopcocks at the battery shelf 180 degrees. The batteries that have absorbed the CO_2 of the aliquot current the preceding day are then closed. Disconnect them from the camshaft of the rocking device and connect the newly filled batteries to it. During the downward movement of aspirator pipette No. 1, watch the air locks in the aliquot air current, especially those at the end, to see that the air of the aliquot current bubbles through correctly. Inspect the newly connected collecting tubes for the composite sample to see whether the air enters properly. Read and record the temperature of the aspirator and barometer. (See table 12 in Appendix.)

7:50 a.m. Record the temperature and humidity of the air in the chambers, together with the temperature and velocity of the water in

the condenser. (See table 8 in Appendix.) Record the figures on the motility recorder. (See table 9 in Appendix.) Record the readings of the water meters.

Feed the animals and record the amount of food given.

7:55 a.m. Make a time mark on the paper slip of the recorder for standing and lying. Label the sampling bulbs containing the momentary air samples and carry them into the gas-analysis room. Transfer the composite air samples from the collecting tubes to sampling bulbs; label them and carry them into the room for gas analysis.

A technician analyzes the four gas samples (see table 16 in Appendix); he then titrates the four absorbing batteries (see tables 14 and 15 in Appendix), fills them with fresh alkali solution, and puts them on the cradles again. Finally, he connects them to the duct of the aliquot air current so that the next morning the mere turning of the six stopcocks, as mentioned before, directs the air of the aliquot current through these batteries.

This analytical work requires almost the full time of a technician, who does the calculation of the results during the weeks when there is no trial.

Each day feces and urine are removed from the separating device and measured. Aliquot samples of the urine are drawn. The carbon in them is determined by wet combustion with K_2CrO_4; the nitrogen by the Kjeldahl method. The feces are dried and analyzed for nitrogen (Kjeldahl), ether extract, crude fiber (Weende), and energy by direct combustion in the calorimetric bomb. The wet combustion, originally used for all carbon determination, has been replaced, in the cases of food and feces, by the absorption and titrimetric determination of the CO_2 from the calorimetric bomb after the determination of the heat of combustion.

The drying of the urine and the combustion in the calorimetric bomb have not proved satisfactory, because too great a loss occurs during the drying process and because the combustion is in many cases incomplete. Another difficulty is that the urine may contain preformed CO_2, produced by the decomposition of urea or other constituents after leaving the body. The calorimetry of the dried urine, therefore, even if it can be made satisfactorily, does not necessarily give a reliable result for the amount of energy lost by the animal as urine. Several preliminary tests made here confirm the result of Kellner and Köhler (1900), who found that the energy content of the urine may be calculated from its carbon content.

From thirty-six results obtained by Kellner and Köhler on the relation of carbon content and heat of combustion of urine of steers under various conditions of feeding, the author has calculated that 1 gram

carbon in urine of steers corresponds to 10 ± 0.035 Cals. The standard deviation of the single result for this mean was ± 0.21 Cals.; the coefficient of variability was thus ± 2.1 per cent.

This relation is used to determine the energy content of the urine indirectly on the basis of its carbon content, which may be measured by wet combustion without drying the samples.

Calculation of Results

Carbon Dioxide Production of the Animal.—The amount of CO_2 produced by the animal is the difference between that which has left the chamber and that which has entered, plus the difference between the amounts in the chamber at the end and at the beginning.

CO_2 produced = CO_2 leaving chamber − CO_2 entering chamber
+ (CO_2 in chamber at end − CO_2 in chamber at the start),

or

$$(CO_2)_p = (CO_2)_u - (CO_2)_i + (CO_2)_e - (CO_2)_a \qquad (28)$$

The amount of CO_2 leaving the chamber $(CO_2)_u$ is calculated by multiplying the concentration of the CO_2 in the composite sample (gas analysis) by the amount of air that left the chamber (see table 18), or by multiplying the amount of CO_2 found in the aliquot air current (titration) by the ratio of the amount of air in the total air current to that in the aliquot current. (See table 17 in Appendix.)

If c_c means the concentration of CO_2 in the composite sample of the air leaving the chamber and L_{us} denotes the amount of air, in liters, that has left the chamber in that period, the amount of CO_2, in liters, $(CO_2)_{us}$ that has left the chamber is

$$(CO_2)_{us} = c_c L_{us}. \qquad (29)$$

Similarly, the amount of CO_2 that entered the chamber is

$$(CO_2)_{is} = c_i L_{is} \qquad (30)$$

where c_i = concentration of CO_2 in the air entering the chamber, and

L_{is} = amount of air entering the chamber (liters at standard conditions).

The amount of air entering the chamber equals the amount taken out only if the respiratory quotient of the animal in the chamber is unity—that is, if each liter of O_2 absorbed is replaced by one liter of CO_2 given off. In general, the amount of air entering the chamber differs from the amount flowing out. The largest difference occurs at the respiratory quotient for fat combustion, 0.7, in which case only seven-tenths of the

absorbed O_2 is replaced by CO_2. If, at a respiratory quotient of 0.7, the difference between the amount of CO_2 leaving the chamber and that entering is 1.0 per cent of the amount of air leaving, the difference between the amount of O_2 entering the chamber and leaving it will be $\frac{1.0}{0.7}$ or 1.4 per cent of the amount of air leaving. (Amounts are given in volumes at standard conditions.) The amount of air entering the chamber is in this case 100.4 per cent of the amount leaving, provided that the amount in the chamber remains constant.

Since, however, the concentration of the CO_2 in the inflowing air is only 0.03 per cent and is subject to an error of $\pm$ 10 per cent of this value, the difference between the amounts of inflowing and outflowing air, introducing a maximal error of 0.4 per cent, is not to be considered for the calculation of the CO_2 production of the animal. It is thus correct to formulate

$$(CO_2)_{is} = c_i L_{us} \qquad (31)$$

The CO_2 content of the chamber at the end, $(CO_2)_{es}$, is the product of the concentration and the volume reduced to standard conditions, V_{es}. Thus

$$(CO_2)_{es} = c_e V_{es} \qquad (32)$$

where c_e=concentration of CO_2 in the momentary air sample taken at the end of the period and V_{es}=air volume in the chamber at the end reduced to standard conditions.

Similarly, the CO_2 content of the chamber at the start is calculated as follows:

$$(CO_2)_{as} = c_a V_{as} \qquad (33)$$

where c_a=concentration of CO_2 in the momentary sample at the start and V_{as}=air volume in the chamber at the start reduced to standard conditions. (See table 19 in Appendix.)

The amount of CO_2 (in liters at standard conditions) produced in a certain period $(CO_2)_{ps}$, is thus

$$(CO_2)_{ps} = L_{us}(c_c - c_i) + c_e V_{es} - c_a V_{as}. \qquad (34)$$

If the simplification $(CO_2)_i = c_i L_{us}$ is introduced into Möllgaard's equation for the calculation of the CO_2 (Möllgaard, 1929, p. 71) and if the correction for the manger is neglected, Möllgaard's is identical with our equation. (See table 23 and 24 in Appendix.)

Methane Production.—As the CH_4 content in the inflowing air may be neglected, the CH_4 production, in terms of volume at standard conditions, may be calculated according to the equation

$$(CH_4)_{ps} = (CH_4)_{bs} \times R \tag{35}$$

where $(CH_4)_{ps}$ = amount of CH_4 produced in liters$_s$

$(CH_4)_{bs}$ = amount of CH_4 determined by titration in battery in liters$_s$

$$R = \frac{\text{volume}_s \text{ of air in main current}}{\text{volume}_s \text{ of air in aliquot current}}.$$

The change of the CH_4 content in the chamber is neglected. (See table 17 in Appendix.)

Oxygen Consumption.—The amount of O_2 consumed, O_{ps} (expressed as volume at standard conditions) is calculated as the difference between the amount of O_2 entering, O_{is}, and the amount of O_2 leaving the chamber, O_{us}, plus the difference between the amount of O_2 in the chamber at the start, O_{as}, and the amount of O_2 there at the end of the period, O_{es}.

$$O_{ps} = O_{is} - O_{us} + O_{as} - O_{es} \tag{36}$$

In calculating the amount of O_2 entering the chamber, one cannot neglect the difference between the amount of entering air, L_{is}, and outflowing air, L_{us}.

The amount of air flowing into the chamber may be calculated from that leaving, on the basis of the assumption that the quantity of N_2 breathed in by the animal is the same as that breathed out. According to this assumption,[9]

$$N_{is} + N_{as} = N_{us} + N_{es} \tag{37}$$

or

$$N_{is} = N_{us} + N_{es} - N_{as}.$$

The amount of air entering the chamber is given by the following equation:

$$L_{is} - \frac{N_{is}}{n_i} = \frac{N_{us} + N_{es} - N_{as}}{n_i}. \tag{38}$$

The amount of O_2 entering the chamber, O_{is}, consequently is

$$O_{is} = o_i \times L_{is} = \frac{o_i}{n_i} \times N_{is} = \frac{o_i}{n_i}(N_{us} + N_{es} - N_{as}) \tag{39}$$

The other three terms of equation 35 expressed in volumes at standard conditions, O_{us}, O_{as}, O_{es}, are calculated in the same way as the corresponding terms for CO_2. Thus the equation for the amount of O_2 consumed is

$$O_{ps} = \frac{o_i}{n_i}(N_{us} + N_{es} - N_{as}) - o_u \times L_{us} + o_a \times V_{as} - o_e \times V_{es} \tag{40}$$

[9] The symbols used in equations 37 to 39 are defined after equation 40.

where

O_{ps} = amount of oxygen consumed in liters at standard conditions

o_i = concentration of oxygen in the inflowing air (volume of O_2 per unit of volume of air)

n_i = concentration of nitrogen in the inflowing air

N_{us} = amount of nitrogen leaving the chamber in liters at standard conditions

N_{es} = amount of nitrogen in the chamber at the end in liters at standard conditions

N_{as} = amount of nitrogen in the chamber at the start in liters at standard conditions

o_u = concentration of oxygen in the composite sample of the outflowing air (liters O_2 per liter of air)

L_{us} = amount of air which, during the period, left the chamber in liters at standard conditions

o_a, o_e = concentration of oxygen in the momentary sample taken at the start (a) and the end (e) respectively.

V_{as},V_{es} = volume of air in the chamber reduced to standard conditions at the start (a) and the end (e) respectively.

This equation for calculating the oxygen consumption is applicable also in cases where a difference in the N_2 content of the chamber at the start and the end has to be considered. Möllgaard's similar equation (Möllgaard, 1929, p. 71) seems to be based upon the assumption that such a difference in the N_2 content of the chamber is negligible, which is correct for all trials of long duration. A possible difference in the N_2 content of the chamber between start and end is, furthermore, decreased by running the apparatus several hours before the real experiment starts.

Correction for Opening the Chamber for Feeding.—The room in front of each chamber, which serves as an air lock for the feedbox, has a volume of 460 liters. If the air in this room is assumed to have the same composition as that in the chamber and if after the outside cover is opened and the food is put in, the air is assumed to be the same as that outside, then the opening has the same effect as if 460 liters of air—approximately the amount of one period of the aspirator—had been sucked from the chamber in addition to the normal ventilation. The normal ventilation in 24 hours is $492 \times 0.458 = 225$ cu. m of air (at room temperature). Therefore $\frac{0.46 \times 100}{225} = 0.20$ per cent should be added to the amount of air sucked from the chamber for each opening of the feedbox in 24 hours.

For the 24-hour trial, the result (CO_2 production and O_2 consumption) at given figures for the composition of the air is approximately proportional to the amount of air sucked from the chamber, and therefore the correction of 0.2 per cent for each opening of the feedbox in 24 hours may also be applied directly to the figure for CO_2 production and O_2 consumption.

Calculation of the Carbon Balance.—In addition to the analysis of food and excrements as carried out for ordinary digestion trials, the carbon content is determined in feed, feces, and urine. (See pp. 50–51). These data, in connection with the results of the CO_2 production, are used for calculating the total income and output of carbon. For these calculations the densities of CO_2 and CH_4 have been calculated from that of O_2, assuming that the CO_2 and the CH_4 in the dilution in which they occur in respiration trials behave as ideal gases (see p. 11). Thus air contains 0.5359 gram C for each liter of CO_2 or of CH_4 it contains. (See table 27 in Appendix.)

Calculation of the Energy Balance.—The energy balance is calculated from figures for the heat of combustion of feed and feces as determined directly in the calorimetric bomb. The energy content of the urine in kilogram-calories is obtained by multiplying the carbon content in grams by 10 (p. 50). For the energy in CH_4, the number of grams of carbon in CH_4 is multiplied by 17.57. This factor is based on a heat of combustion of 210.8 Cals. per mol CH_4 as given in the critical tables (Anonymous, 1929, p. 163). The difference between the energy in food and that in feces is the digested energy. From it is subtracted the energy in urine and CH_4. The rest is the metabolizable energy. This is the sum of the animal's heat production and the gain of energy as produced body substance. This last figure is negative if the energy given off as heat is larger than the metabolizable energy.

The gain (or loss) of energy is calculated from the carbon balance in connection with the nitrogen balance. One part of the gain of carbon is contained in gain of protein. The gain in protein is calculated by multiplying the gain in nitrogen by 6.25 and the carbon content of the protein gained is calculated by multiplying the gain in protein by 0.52. The carbon in the gained protein is subtracted from the total amount of carbon gained. The rest is the carbon stored as body fat. This figure is to be divided by 0.765 in order to obtain the amount of gained body fat in grams. Each gram of stored protein represents a gain in energy of 5.7 Cals.; each gram of produced body fat, a gain of 9.5 Cals. When the energy of the gained body substance is subtracted from the metabolizable energy, the difference is the heat production of the animal.

The procedure of this calculation is illustrated in table 27 (p. 70).

Accuracy

Test with CO_2 Entering from Outside.—From nineteen consecutive tests with CO_2 entering the chamber from a bomb, the quantity measured by a wet gas meter, four tests had to be omitted for known reasons, as follows: the mercury valve of the sampling device was out of order; the electric current was interrupted; the fan inside the chamber was not operating; and an error occurred in the reading of the wet gas meter. From the remaining fifteen tests on the average 0.16 per cent less CO_2 was found by means of the gas-analysis method than has been introduced into the chamber according to the readings of the wet gas meter.

The average deviation between the results of the wet gas meter for the introduced CO_2 and the amount determined by the experiment with gas analysis is $\pm$ 1.55 per cent of the mean amount of CO_2 measured. The square root of the mean square deviation is $\pm$ 1.84 per cent of this mean.

Test with Alcohol.—The accuracy of the apparatus has also been tested by combustion in the chambers of a known amount of C_2H_5OH. In eight trials without regard to the combustible products in the air, an average of 1.085 $\pm$ 0.252 per cent less CO_2 has been found than was expected. In six trials in which the CO_2 of the combustible products in the air had been determined and added to the result, 0.70 $\pm$ 0.67 per cent more CO_2 was found than was expected. The standard deviation of one determination was $\pm$ 1.15 per cent. The average difference between the result of the gas analysis and that of the absorbing battery irrespective of the sign was 1.3 per cent of the result. The R.Q. in thirteen alcohol trials was, on the average, 0.663 $\pm$ 0.002. (The O_2 determination for one trial was lost by an accident with a sampling bulb during gas analysis).

SUMMARY

The measurement of the heat production of the animals (direct calorimetry) in connection with their carbon balance is necessary for certain fundamental studies on the relation between energy metabolism and biochemical processes. For research on most agricultural problems, the measurement of the carbon and nitrogen balances, with or without the determination of the oxygen consumption, is sufficient.

The measurement of the carbon and nitrogen metabolism alone is, for this kind of research, preferable to the measurement of the energy metabolism alone, because from the carbon and nitrogen balances the energy balance may be calculated (indirect calorimetry). The reverse calculation is not possible.

In connection with agricultural research, the measurement of the CO_2 production of the animal (as an integral part of the carbon balance) is more important than the determination of the O_2 consumption.

It is, however, advantageous to know the respiratory quotient, that is, the volume of CO_2 produced per unit of volume of O_2 consumed. The formulas for calculating the partition of the metabolized carbon and energy between fat and carbohydrates on the basis of the nonprotein respiratory quotient are developed, and the results given in tables.

The respiration apparatus at the University of California combines the principles of Pettenkofer and Tigerstedt for an *open-air-current-enclosure* apparatus.

A double chamber makes it possible to carry out pair trials—that is, to measure simultaneously the metabolism of two animals which differ but slightly except for the one variable which is under investigation.

A window in the central partition between the two chambers, allowing the animals to see each other, adds to the comfort and, consequently, the normal behavior of the animals.

The chamber has been made as small as possible without interfering with the comfort of the cows.

The apparatus is equipped with a device for feeding the animals and also for admitting an attendant without interrupting the experiment.

An automatic device for separating feces and urine (system of Ritzman) is installed.

A device for controlling temperature and humidity inside the chamber is installed. It consists of a condensing coil in an air channel above the cow with a fan to drive the air through this channel and over a set of electric heaters controlled by a specially designed device.

The rate of ventilation is adjustable; it is ordinarily maintained at 200 cubic meters per 24 hours for each chamber.

The air is sucked out of the chamber and driven to outside by means of four pipettes (two per chamber) of 225 liters capacity, made of sheet copper and alternately immersed and withdrawn from water in a tank. The two air valves connected with each pipette and their automatic operation are described; also the device for reversing the motor.

A regulator for maintaining a slight suction inside is installed on each chamber.

The device for taking the air samples is described. For each cycle of the aspirator, when 450 liters of air are sucked into the aspirator from each chamber, a sample of 1 cc and one of 20 cc are taken over mercury.

The 1-cc sample is driven as the composite sample to a collecting device, which is so constructed as to cause no differences in pressure during the collection of the gas.

The 20-cc samples are driven over a battery with $Ba(OH)_2$ solution, so designed that the gas does not bubble through the solution; consequently no back pressure occurs. A high efficiency of absorption is obtained by rocking the battery.

The air sample leaving the battery is driven over a red glowing wire in a combustion tube for the oxidation of CH_4. The produced CO_2 is absorbed in a second system of absorbing batteries.

Devices for recording the motility and the position of the cows are installed, together with an absorbing device for the NH_3 produced in the chamber. A device for making an alarm if the suction stops for more than 1 minute is installed on each chamber; other devices also give alarm if the temperature in the chamber varies too greatly, or if the flow of brine is interrupted.

The operation of the chamber is described, and the calculation of the results explained.

The standard difference between the amount of CO_2 introduced into the chamber, as measured by a wet gas meter, and the result obtained in the Tigerstedt system (gas analysis) has been found to be $\pm$ 1.55 per cent. The result of the Pettenkofer system (absorbing battery) checked that of the Tigerstedt system with a standard difference of $\pm$ 1.7 per cent. The standard deviation for the mean result in fourteen alcohol tests was $\pm$ 1.15 per cent; the R.Q. was 0.663 $\pm$ 0.002.

ACKNOWLEDGMENTS

It has been my privilege to visit some of the most important laboratories for metabolic research and enjoy valuable discussions with A. Krogh and H. Möllgaard, of Copenhagen, Denmark; M. Steuber, of Berlin; G. Fingerling, of Leipzig, Germany; F. G. Benedict, T. M. Carpenter, and E. Fox, of Boston, Massachusetts; E. G. Ritzman, of Durham, New Hampshire; P. E. Howe, of Washington, D. C.; E. B. Forbes, M. Kriss, and R. Swift, of State College, Pennsylvania; H. H. Mitchell, of Urbana, Illinois; and S. Brody, of Columbia, Missouri.

The idea of "pipetting" the air may be traced back to discussions with my friend and former mechanic at the Swiss Institute of Animal Nutrition in Zurich, A. Wirth.

I wish to acknowledge the friendly coöperation of members of the staff of the College of Agriculture at Davis, in particular the ever encouraging help of G. H. Hart, who originated the plan to provide the Division of Animal Husbandry at Davis with the facilities for studies of the respiratory exchange in large animals.

The chamber was built by R. Underhill, carpenter at the University Farm. For analytical work I am indebted to Helene K. Rohwer, R. W. Caldwell, W. Campbell, and H. Johnson; for help in operating and improving the machine, to E. Pfanner.

LITERATURE CITED

Anonymous.
1929. Heats of combustion of organic compounds. International Critical Tables 5:162–169.

Armsby, H. P.
1904. The respiration calorimeter at the Pennsylvania Experiment Station. Exp. Sta. Record 15:1037–1050.
1908. Principles of animal nutrition. 3d ed. 614 p. John Wiley and Sons, New York.
1922. The nutrition of farm animals. 743 p. Macmillan Co., New York.

Atwater, W. O., and F. G. Benedict, *et al.*
1899. Experiments on the metabolism of matter and energy in the human body. U. S. Dept. Agr. Office of Exp. Sta. Bul. 69:1–112.
1902. Experiments on the metabolism of matter and energy in the human body, 1898–1900. U. S. Dept. Agr. Office of Exp. Sta. Bul. 109:7–147.
1903. Experiments on the metabolism of matter and energy in the human body, 1900–1902. U. S. Dept. Agr. Office of Exp. Sta. Bul. 136:9–357.

Benedict, F. G.
1926. Methoden zur Bestimming des Gaswechsels bei Tieren und Menschen. Abderhaldens Handbuch der biologischen Arbeitsmethoden. Abtl. 4. Teil 10:415–674.

Benedict, F. G., and E. G. Ritzman.
1923. Undernutrition in steers. Carnegie Inst. Wash. Pub. 324:1–333.
1927. The metabolism of the fasting steer. Carnegie Inst. Wash. Pub. 377: 1–245.

Borsook, H., and H. M. Winegarden.
1930. The free energy of glucose and of tripalmitin. Natl. Acad. of Sci. Proc. 16:559–573.

Brody, S.
1928. Energy and nitrogen metabolism during the first year of postnatal life. Missouri Agr. Exp. Sta. Research Bul. 143:6–18.

Capstick, J. W.
1926. Ein Calorimeter für das Arbeiten mit grossen Tieren. Abderhaldens Handbuch der biologischen Arbeitsmethoden Abt. 4, Teil 10:793–816.

Carpenter, T. M.
1921. Tables, factors, and formulas for computing respiratory exchange and biological transformations of energy. Carnegie Inst. Wash. Pub. 303:1–123.

Chwolson, O. D.
1922. Lehrbuch der Physik. 2d ed. III, 1. 450 p. Friedrich Viehweg und Sohn, Braunschweig, Germany.

Grafe, E.
1926. Quantitative Bestimmung des Gasstoffwechsels mittels Pettenkofer-Tigerstedt, Jacquet und Benedict Apparaten. Abderhaldens Handbuch der biologischen Arbeitsmethoden, Abt. 4, Teil 10:309–414.

Gregorovius, P.
1927. Entgasung bei hoher Temperatur. Hütte 25th ed. 4:687–714.

Kellner, O.
1919. Die Ernährung der landwirtschaftlichen Nutztiere. 8 Aufl. 667 p. Herausgegeben von G. Fingerling. Paul Parey, Berlin.

Kellner, O., and A. Köhler.
1900. Untersuchungen über den Stoff- und Energiewechsel des erwachsenen Rindes bei Erhaltungs und Produktionsfutter. Landw. Vers. Sta. 53:1–474.

Kleiber, M.
1928. Wie und mit welcher Genauigkeit kann man die Schwankungen der Respirationsintensität ohne Tracheotomie oder Mundstück bestimmen. Pflüger's Arch. Physiol. 220:599–605.
1932. Body size and metabolism. Hilgardia 6(11):315–353.
1933*a*. Contribution to the method of gas analysis for respiration trials. Jour. Biol. Chem. 101:583–594.
1933*b*. Relation between volume of respiration chamber and concentration of carbon dioxide in end sample and in composite sample of air. Indus. and Engin. Chem. 5:98–100.

Kolthoff, I. M., and H. Menzel.
1928. Volumetric analysis. Tr. by N. H. Furman. Vol. 1, The theoretical principles. 289 p. Vol. 2, Practical methods. 552 p. John Wiley and Son, New York.

Krogh, A., and M. Krogh.
1913. A study of the diet and metabolism of Eskimos undertaken in 1908 on an expedition to Greenland. Meddelelsen om Gröuland 51:1–52.

Krogh, A.
1916. The respiratory exchange of animals and man. 173 p. Longmans, Green & Co., London and New York.

Kühn, G., *et al.*
1894. Fütterungs- und Respirationsversuche mit volljährigen Ochsen. Landw. Vers. Sta. 44:257–581.

Loewy, A.
1926. Der respiratorische und der Gesamtumsatz. Oppenheimers Handbuch der Biochemie. 2nd ed. 6:125–278. G. Fischer, Jena.

Möllgaard, H.
1929. Fütterungslehre des Milchviehs. Die quantitative Stoffwechselmessung und ihre bisherigen Resultate beim Milchvieh. 246 p. M. and H. Schaper, Hanover, Germany.

Möllgaard, H., and A. C. Anderson.
1917. Respirationsapparatet, dets Betydning og Anvendelse ved rationelle Forsög over Hornkvaegets Maelkeydelse [Respiration apparatus: its significance and use in experiments with dairy cattle]. Beret Forsøgslab. K. Vet. og Landbohøjskoles [Denmark] 94:1–180.

Pettenkofer, M.
1862. Über die Respiration. Ann. d. Chemie u. Pharm. 2d supplement, p. 1–52.

Petterson, O.
1886. Luftanalyse nach einem neuen Prinzip. Zeitschr. f. analyt Chem. 25:467–478.

Pickering, S. F.
1928. P-V-T relations in the gaseous state for substances which are gases at 0° and 1 atmosphere. International Critical Tables 3:3–17.

Ritzman, E. G., and F. G. Benedict.
1929. Simplified technique and apparatus for measuring energy requirements of cattle. New Hampshire Agr. Exp. Sta. Bul. 240:1–30.

Rubner, M.
1894. Die Quelle der tierischen Wärme. Zeitschr. Biol. 30:73.

Sondén, K., and R. Tigerstedt.
1895. Untersuchung über die Respiration und den Gesammtstoffwechsel des Menschen. Skand. Arch. Physiol. 6:1–223.

Williams, H. B., J. A. Riche, and G. Lusk.
1912. Metabolism after meat ingestion. Jour. Biol. Chem. 12:349–376

Wolpert, H.
1896. Über den Einfluss der Lufttemperatur auf die im Zustand anstrengender körperlicher Arbeit ausgeschiedenen Mengen Kohlensäure und Wasserdampf beim Menschen. Arch. Hyg. 26:32–67.

Zuntz, N.
1926. Beziehungen zwischen Nährstoffen und Leistungen des Körpers. Oppenheimers Handbuch der Biochemie 2d ed. 6:411–457.

Zuntz, N., and Schumburg.
1901. Studien zur Physiologie des Marsches. Bibliothek von Coler 6:1–361. Hirschwald, Berlin.

APPENDIX

Records and Calculations of Results of a Respiration Trial[10]

The following tables illustrate the method used at this Station for recording the data and calculating the results of a respiration trial. The data are taken from an actual experiment (No. PC_2, North Chamber, Hereford heifer No. 29). The two first days of the experiment were chosen as an example. Table 16 shows the procedure of calculating the composition of one gas sample from the readings of a Carpenter apparatus for CO_2 and a Kleiber apparatus for O_2. In tables 10, 11, 26, and 27, however, the data for the whole experimental period are used in order to show the calculation.

TABLE 8

Environmental Conditions

Date: March, 1932		Temperature of air in chamber	Relative humidity of air in chamber	Temperature of condenser water	Velocity of condenser water
day	*hour*	*°C*	*per cent*	*°C*	*liters per min.*
14	7:45	22.0	63	12	4.0
	19:45	20.3	70	10	4.0
15	7:45	18.5	73	9	3.5
	19:45	19.0	68	....	....
16	7:45	18.5	73	10	2.7

TABLE 9

Behavior of Animal

Date: March, 1932		Motility		Position		Remarks
		Reading	Δ	Number of changes	Standing time in per cent of total time	Body weight: 744 pounds
day	*hour*				*per cent*	
14	7:45	2,758				
			146	4	71	
	19:45	2,904				
			111	8	28	
15	7:45	3,015				
			161	11	41	
	19:45	3,176				
			109	7	28	
16	7:45	3,285				

[10] The subscript $t°$ with an air volume or a barometer reading means the volume or the length of the mercury column without temperature correction. The superscript 0° with a barometer reading indicates that this reading has been corrected to 0° C. The subscript s with a term standing for a gas volume indicates that the volume has been reduced to standard conditions: dry, at 0° C and 760-mm mercury pressure.

TABLE 10

Food Consumption*

Date: March, 1932	Food consumed per day		Composition of food per 100 grams dry matter		
	Air dry	Dry matter	Nitrogen	Carbon	Energy (measured)
	pounds	*grams*	*grams*	*grams*	*Cals.*
14 to 26	3	1,207	2,392	43.44	436.1

* Table 10 does not show the daily records but the averages for the whole experimental period because these averages are used in the later calculations.

TABLE 11*

Excreta per Day

Date: March, 1932	Dry matter in feces per day	Composition of feces per 100 grams dry matter			In urine, total per day	
		Nitrogen	Carbon	Energy	Nitrogen	Carbon
	grams	*grams*	*grams*	*Cals.*	*grams*	*grams*
14 to 26	316	2.62	40.42	411.3	26.9	31.0

* Table 11 does not show the daily records but the averages for the whole period because these averages are used in later calculations.

TABLE 12

Ventilation

Volume of aspirator pipettes Nos. 1 and 2 ..... 458.646 liters
Decrease in volume by telescoping per period ..... 10.960 liters
Decrease due to difference in water level in valves ..... 0.216 liters

Total decrease in volume for air intake ..... 11.176 liters

Air volume $t°$ per period of aspirator pipettes Nos. 1 and 2 ..... 447.470 liters

Date: March, 1932		Productimeter reading	Number of aspirator periods	Temperature of aspirator	Barometer		Volume factor	Amount of air leaving chamber	
					read $b_t°$	reduced to 0°C $b_o°+1$ mm		Per period	Total
day	*hour*			°C	*mm Hg*	*mm Hg*		l_s	*cu.m.* s
14	7:45	18,537	490	22.1	758.6 (23°C)	756.8			
	19:45	.....		22.1	760 3 (23°C)	758.5	0.900	402.72	197.333
15	7:45	19,027	489	21.5	763.8 (21°C)	762.2			
	19:45	.....		21.3	765.8 (23°C)	763.9	0.910	407.15	199.096
16	7:45	19,516	.....	20.9	768.7 (21°C)	767.1			

TABLE 13

Amount of Air in Chamber

Volume of chamber.....11.4 cu.m
Volume of animal..... 0.3 cu.m
Volume of air in chamber.....11.1 cu.m

Date: March, 1932		Temperature of air in chamber	Pressure in chamber reduced to 0°C	Humidity			Pressure of dry air in chamber	Volume factor for air in chamber	Amount of dry air in chamber
				Per cent of saturation	Pressure of water vapor at saturation	Pressure of water vapor in chamber			
day	*hour*	*°C*	*mm Hg*	*per cent*	*mm Hg*	*mm Hg*	*mm Hg*		*cu.m.*
14	7:45	22.0	755.8	63	19.8	12.5	743.3	0.905	10.046
15	7:45	18.5	761.2	73	16.0	11.7	749.5	0.924	10.256
16	7:45	18.5	766.1	73	16.0	11.7	754.5	0.929	10.312

TABLE 14

Titration of Liquid in the Absorbing Battery Before Heating

Date: March, 1932	Total time	Battery No.	Set of absorbers	Titration data					Factor for conversion of vol. of 0.2*N* HCl solution to vol.$_s$ of CO_2 gas	Amount of CO_2 absorbed in aliquot air current
				Amount of $Ba(OH)_2$ solution in absorber		Back titration volume of 0.2*N* HCl used for neutralization (pH 8.4)	Amount of 0.2*N* $Ba(OH)_2$ solution neutralized by CO_2 in equivalents of 0.2*N* HCl solution			
				Measured	Calculated to equivalents of 0.2*N* HCl solution		Each set	Total		
day	*hours*			*ml*	*ml*	*ml*	*ml*	*ml*		*ml.*
14–15	24	1	1	50.00	49.90	21.90	28.00	29.32	1.980	58.06
			2	50.00	49.90	48.65	1.25			
			3	15.00	14.97	14.90	0.07			
15–16	24	2	1	50.00	49.90	21.60	28.30	29.60	1.980	58.61
			2	50.00	49.90	48.75	1.15			
			3	15.00	14.97	14.82	0.15			

TABLE 15

Titration of Liquid in the Absorbing Battery After Heating

Date: March, 1932	Total time	Battery No.	Set of absorbers	Titration data					Factor for conversion of vol. of 0.2*N* HCl solution to vol.$_s$ of CO_2 gas	Amount of CO_2 absorbed in aliquot air current
				Amount of $Ba(OH)_2$ solution in absorber		Back titration volume of 0.2*N* HCl used for neutralization (pH 8.4)	Amount of 0.2*N* $Ba(OH)_2$ solution neutralized by CO_2 in equivalents of 0.2*N* HCl solution			
				Measured	Calculated to equivalents of 0.2*N* HCl solution		Each set	Total		
day	*hours*			*ml*	*ml*	*ml*	*ml*	*ml*		*ml.*
14–15	24	5	1	50.00	49.90	48.00	1.90	2.37	1.980	4.69
			2	50.00	49.90	49.70	0.20			
			3	15.00	14.97	14.70	0.27			
15–16	24	6	1	50.00	49.90	48.00	1.90	2.07	1.980	4.10
			2	50.00	49.90	49.80	0.10			
			3	15.00	14.97	14.90	0.07			

TABLE 16

GAS ANALYSIS—APPARATUS: C FOR CO_2; K, FOR O_2; YEAR: 1932; P: 46

Date of analysis	Kind of sample: Experiment	Kind of sample: S, E, C*	Kind of sample: Date of sample: day	Kind of sample: Date of sample: hour	Volume: Reading	Volume: Correction in 10^{-3} units: Vol.	Volume: Correction in 10^{-3} units: H_2O	Volume: Correction in 10^{-3} units: Tot.	Volume: Corrected volume	Difference of corrected volume (Δ)	Per cent correction†: $V_a-100=a$	Per cent correction†: $\frac{\Delta}{100}a=c$	Result per cent: $\Delta-c$	Result per cent: Average	Gas
3/15	PC_2N	C	3/14	7:45	99.901	+ 5	.....	+ 5	99.906	0.632	negligible		0.632		
			3/15	7:45	99.251									0.632	CO_2
					99.250	+24	.....	+ 24	99.274						
		Same (check)			99.909	+ 4	.....	+ 4	99.913	0.631	negligible		0.631		
					99.255										
					99.258	+24	.....	+ 24	99.282						
3/15	PC_2N	C	3/14	7:45	100.091	+ 1	−130	−129	99.962	20.835	−0.038	-8×10^{-3}	20.843		
			3/15	7:45	79.245										
					79.222									20.840	O_2+CO_2
					79.223	+34	−130	− 96	79.127						
		Same (check)			100.197	+ 4	−130	−126	100.071	20.851	+0.071	$+15\times10^{-3}$	20.836		
					79.332										
					79.315									20.208	O_2
					79.315	+35	−130	− 95	79.220						

* S or E=Momentary sample at start or end. C=Composite sample.

† Per cent correction: If V_a stands for the initial volume (in per cent) the result, $R,=\Delta\frac{100}{V_a}$. If $V_a=100+a$, then it follows that $R=\Delta\frac{100}{100+a}$. If further $a=\frac{100c}{\Delta}$, the result is $R=\Delta\frac{100}{100+\frac{100c}{\Delta}}=\frac{\Delta}{1+\frac{c}{\Delta}}$. By multiplication with $\left(1-\frac{c}{\Delta}\right)$ one obtains

$$R=\frac{\Delta\left(1-\frac{c}{\Delta}\right)}{\left(1+\frac{c}{\Delta}\right)\left(1-\frac{c}{\Delta}\right)}=\frac{\Delta\left(1-\frac{c}{\Delta}\right)}{1-\left(\frac{c}{\Delta}\right)^2}$$

Since the original volume V_a is not far from 100, c is generally small compared with Δ and hence $\frac{c}{\Delta}$ is small compared with unity. Therefore $\left(\frac{c}{\Delta}\right)^2$ may be neglected and the result calculated as $R=\Delta-c$.

TABLE 17

AMOUNTS OF CO_2 AND CH_4 LEAVING CHAMBER (CALCULATED FROM BATTERY)

Date: March, 1932	Total time	Ratio of total air current to aliquot air current* corrected for humidity†	Amount of CO_2 absorbed in battery		Amount of CO_2 leaving chamber (battery)	Amount of CH_4 leaving chamber
			Before heating (table 14)	After heating (table 15)		
day	*hours*		ml_2	ml_2	l_2	l_2
14–15	24	2.215×10^4	58.06	4.69	1,286	104
15–16	24	2.215×10^4	58.61	4.10	1,298	91

* Ratio of total air current to aliquot air current $= \frac{\text{Vol. of aspirator pipettes Nos. 1 and 2}}{\text{Vol. of mercury pipette } AC_1} = \frac{447.47\times10^3 \text{ ml}}{20.045 \text{ ml}} = 2.232\times10^4$.

† Correction for humidity: Air in aspirator is saturated, air in aliquot current only to 69 per cent.

H_2O tension in air of aspirator at 20.3°C = 17.9 mm Hg
H_2O tension in air of aliquot current at 20.3°C = $17.9\times0.69=12.4$ mm Hg
Pressure of dry air in total air current = 759.2−17.9=741.3 mm Hg
Pressure of dry air in aliquot air current = 759.2−12.4=746.8 mm Hg

The volume ratio of 2.232×10^4 is to be multiplied by $\frac{741.3}{746.8}=0.9926$ in order to obtain the ratio of the amounts of dry air in total and in aliquot current. This ratio is thus $2.232\times10^4\times0.9926=2.215\times10^4$. (In recent trials the air in the sampling device has been dry.)

TABLE 18

AMOUNT OF CO_2 AND O_2 LEAVING CHAMBER (GAS ANALYSIS)

Date: March, 1932	Total time	Amount of air leaving chamber (table 12)	Analysis of composite air sample		Amount of CO_2 leaving chamber (gas anal.)	Amount of O_2 leaving chamber
			CO_2	O_2		
day	*hours*	$cu.m_2$	*per cent*	*per cent*	l_2	l_2
14–15	24	197.333	0.632	20.208	1,247	39,877
15–16	24	199.096	0.647	20.244	1,288	40,320

TABLE 19

AMOUNT OF CO_2 AND O_2 IN CHAMBER

Date: March, 1932		Amount of dry air in chamber (table 13)	Analysis of momentary air sample		Amount of CO_2 in chamber	Amount of O_2 in chamber	Increase in amount of CO_2 in chamber (end-start)	Decrease in amount of O_2 in chamber (start-end)
			CO_2	O_2				
day	*hour*	$cu.m_2$	*per cent*	*per cent*	l_2	l_2	l_2	l_2
14	7:45	10.046	0.449	20.404	45	2,050		
							15	−30
15	7:45	10.256	0.583	20.297	60	2,080		
							0	−15
16	7:45	10.312	0.585	20.318	60	2,095		

TABLE 20

AMOUNT OF N_2 LEAVING CHAMBER

Date: March, 1932	Total time	Amount of O_2 leaving chamber (table 18)	Amount of CO_2 leaving chamber (table 18)	Amount of CH_4 leaving chamber (table 17)	Sum of amounts of O_2, CO_2 and CH_4 leaving chamber	Amount of air leaving chamber (table 12)	Amount of N_2 leaving chamber
day	*hours*	l_2	l_2	l_2	l_2	l_2	l_2
14–15	24	39,877	1,247	104	41,228	197,333	156,105
15–16	24	40,320	1,288	91	41,699	199,096	157,397

TABLE 21

AMOUNT OF N_2 IN CHAMBER (CH_4 NEGLECTED)

Date: March, 1932		Amount of CO_2 and O_2 in chamber (table 19)	Amount of air in chamber (table 13)	Amount of N_2 in chamber	Increase in amount of N_2 in chamber (end-start)
day	*hour*	l_2	l_2	l_2	l_2
14	7:45	2,095	10,040	7,951	
					165
15	7:45	2,140	10,256	8,116	
					41
16	7:45	2,155	10,312	8,157	

TABLE 22

AMOUNTS OF O_2 AND CO_2 ENTERING CHAMBER*

Date: March, 1932	Total time	Amount of N_2 leaving chamber (table 20)	Increase of amount of N_2 in chamber (end-start) (table 21)	Amount of N_2 entering chamber	Amount of O_2 entering chamber (N_2 entering $\times$ 0.26490)	Amount of CO_2 entering chamber (N_2 entering $\times 0.38 \times 10^{-2}$)
day	*hours*	l_2	l_2	l_2	l_2	l_2
14–15	24	156,105	165	156,270	41,405	59
15–16	24	157,397	41	157,438	41,714	60

* Premise: Amount of N_2 gas taken in by animal = amount of N_2 gas given off.
Basis: Composition of outdoor air constant: 0.030 per cent CO_2 and 20.940 per cent O_2 (Benedict, 1926, p. 638.)

TABLE 23

CO_2 Production of the Animal Determined by Gas Analysis

Date: March, 1932	Total time	Amount of CO_2 leaving chamber (table 18)	Amount of CO_2 entering chamber (table 22)	Increase of amount of CO_2 in air current	Increase of amount of CO_2 in chamber (end-start) (table 19)	Amount of CO_2 produced by animal (gas analysis)
day	*hours*	l_s	l_s	l_s	l_s	l_s
14–15	24	1,247	59	1,188	15	1,203
15–16	24	1,288	60	1,228	0	1,228

TABLE 24

CO_2 Production of the Animal Determined by Battery

Date: March, 1932	Total time	Amount of CO_2 leaving chamber (table 17)	Amount of CO_2 entering chamber (table 22)	Increase in amount of CO_2 in air current	Increase in amount of CO_2 in chamber (table 19)	Amount of CO_2 produced by animal
day	*hours*	l_s	l_s	l_s	l_s	l_s
14–15	24	1,286	59	1,227	15	1,242
15–16	24	1,298	60	1,238	0	1,238

TABLE 25

O_2 Consumption and Respiratory Quotient

Date: March, 1932	Total time	Amount of O_2 entering chamber (table 22)	Amount of O_2 leaving chamber (table 18)	Decrease in amount of O_2 in air current	Decrease in amount of O_2 in chamber (table 19)	Amount of O_2 consumed by animal	Amount of CO_2 produced by animal (table 23)	R.Q. $= \frac{\text{vol. } CO_2}{\text{vol. } O_2}$
day	*hours*	l_s	l_s	l_s	l_s	l_s	l_s	
14–15	24	41,405	39,877	1,528	−30	1,498	1,203	0.803
15–16	24	41,714	40,320	1,394	−15	1,379	1,228	0.890

TABLE 26

Respiratory Exchange: Summary

Date: March, 1932	Production of CO_2 determined by		Production of CH_4 (table 17)	Consumption of O_2 (table 25)	R.Q. (table 25)
	Gas analysis (table 23)	Battery (table 24)			
day	l_s	l_s	l_s	l_s	
14–15	1,203	1,242	104	1,496	0.803
15–16	1,228	1,238	91	1,399	0.890
16–17	1,185	1,198	91	1,329	0.891
17–18	1,143	1,173	18	1,264	0.904
18–19	1,170	1,182	87	1,265	0.924
21–22	1,058	1,063	39	1,164	0.909
22–23	1,147	1,169	50	1,350	0.850
23–24	1,106	1,056	29	1,281	0.863
24–25	1,208	1,223	79	1,437	0.841
25–26	1,189	1,191	66	1,415	0.840
Mean	1,164	1,174	65	1,340	0.871
Standard deviation of mean	±17	±20	± 9.5	±31	±0.012
Standard deviation of single result	±52	±65	±30	±99	±0.037
Coefficient of variability, in per cent	± *4.5*	± *5.6*	±*46*	± 7.4	±*4.3*

TABLE 27

N, C, and Energy Balance per Day

March 14-26, 1932	N		C		Energy	
	In	Out	In	Out	In	Out
	grams	*grams*	*grams*	*grams*	*therms**	*therms**
In food 3 pounds air dry	28.9		524		5.26	
In feces		8.3		128		1.30
Digested	20.6		396		3.96	
In urine		26.9		31		0.31†
In methane (65 l_s CH_4)				35‡		0.62§
Metabolizable		−6.3	330		3.03	
Respiration (heat) 1,164 l_s CO_2				624¶		6.65¶¶
Net (change in body substance)		−6.3	−294			−3.62§§
Specifically:						
Loss of body protein (N_{net}×6.25= 39 grams)		6.3		20‖		0.22**
Loss of body fat $\frac{\text{C net}-\text{C protein}}{0.765}$=358 grams		6.3		274††		3.40‡‡

* 1 therm=1,000 kilogram-calories.

† Calories in urine=grams C in urine×10.

‡ 1 liter$_s$ CH_4=0.536 gram C.

§ Calories in CH_4=C in CH_4×17.57.

¶ 1 liter$_s$ CO_2=0.536 gram C.

‖ C in protein=N_{net}×6.25×0.52=N_{net}×3.25.

** Calories in protein=N_{net}×6.25×5.7=N_{net}×35.6

†† C in fat=C_{net}−C in protein.

‡‡ Calories in fat=grams fat×9.5=C in fat×12.42.

§§ Total net energy=energy in body fat+energy in body protein.

¶¶ Heat production=metabolizable energy−net energy.